PHYSICAL CHARACTERIZATION OF CHITOSAN FLOCS AND ASSESSMENT OF MICROBIAL REDUCTIONS WITH THE USE OF CHITOSAN ACETATE AS A CLOTH FILTER AID

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ABSTRACT

Hemali H. Oza: Physical Characterization of Chitosan Flocs and Assessment of Microbial Reductions with the use of Chitosan Acetate as a Cloth Filter Aid
(Under the direction of Mark D. Sobsey)

The World Health Organization estimates 2.1 billion people lack access to safely managed water. Cloth filtration is often employed in rural and developing communities within South Asia for point-of-use water treatment, but bacteria and viruses are too small for efficient removal by this method. Chitosan is a biodegradable, cationic, organic polymer derived from chemical treatment of chitin. Chitosan acts as a coagulant to agglomerate contaminant particles in water, thereby facilitating filtration of contaminants. This research 1) evaluated the use of chitosan acetate as a pre-treatment coagulation process followed by cloth filtration and 2) assessed floc particle size in three stirring conditions. *E. coli* KO11 bacteria and MS2 coliphage virus removals were quantified using culture-based methods. Chitosan acetate pre-treatment, followed by cloth filtration, meets the protective (2-star) WHO performance level for bacterial and viral reductions, and effluent turbidity is consistently reduced to < 1 NTU, meeting U.S. EPA and WHO targets.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AA	Awesome Agar
BSF	BioSand Filter
CFU/mL	Colony forming unit/milliliter
CWF	Ceramic water filters
DAL	Double Agar Layer
DALY	Disability-Adjusted Life Year
DD	Degree of deacetylation
DLS	Dynamic Light Scattering
DOC	Dissolved organic carbon
EPA	Environmental Protection Agency
GlcNAc	N-acetyl-D-glucosamine
GlcN	D-glucosamine
HICs	High Income Countries
HWTS	Household Water Treatment Systems
JMP	Joint Monitoring Program
LMICs	Low- and Middle-Income Countries
LRV	Log_{10} Reduction Value
MDG	Millennium Development Goal
mg/L	Milligram per liter
NTU	Nephelometric Turbidity Unit
OWASA	Orange Water and Sewer Authority
PDA	Photometric Dispersion Analyzer

PFU	Plaque-forming units
POU	Point-of-Use
PSA	Particle Size Analyzer
QMRA	Quantitative Microbial Risk Assessment
rpm	Revolutions per minute
SDG	Sustainable Development Goal
SLS	Static Light Scattering
SODIS	Solar Disinfection
TSB	Tryptic Soy Broth
UV	Ultraviolet
UNICEF	United Nations International Children's Emergency Fund
WaSH	Water, sanitation and hygiene
WHO	World Health Organization

CHAPTER 1: INTRODUCTION

According to the World Health Organization (WHO), approximately 2.1 billion people lacked safely managed drinking water in 2015 (**Table 1.1**). Safely managed water is defined as drinking water collected from improved water sources that is also located on the premise and free of fecal and priority contaminants (WHO & UNICEF, 2017b). Rural and developing populations are less likely to have access to improved drinking water sources, as evidenced by the 90% of 884 million people living without access to improved drinking water who are located in rural and developing regions (WHO & UNICEF, 2017a; WHO, 2014a). Contaminated water can spread infectious diseases, including diarrhea and other gastrointestinal illnesses. These diseases are caused by a variety of different enteric pathogens, including protozoan parasites such as *Entamoeba hystolytica*, *Cryptosporidium parvum* and *hominis*, *and Giardia lamblia*; bacteria such as *Escherichia coli*, *Salmonella* spp., *Salmonella typhi*, the cause of Typhoid fever, *Vibrio cholera*, the cause of cholera; and viruses such as hepatitis A and E viruses that cause infectious hepatitis as well as noroviruses rotaviruses and astroviruses that cause diarrheal and gastrointestinal illnesses (Kotloff et al., 2012). The lack of access to improved drinking water sources leaves developing and rural communities at a greater risk of being exposed to waterborne diseases (WHO & UNICEF, 2015).

Even access to improved water sources in developing and rural areas may not be safely managed, as access does not always equate to absence of absence of pathogens and adverse health risks. A study in Cambodia and Vietnam found improved water sources contaminated with *Escherichia coli* at concentrations ranging from 1 to more than 10^3 CFU/100 mL (Shaheed *et al.*,

2014). The Sustainable Development Goals (SDGs), continue to highlight the gap in service by calling for "universal and equitable access to safe and affordable drinking water by 2030," (WHO, 2019). A systematic review and meta-analysis of 319 studies, reporting on over 90,000 water samples, found over a quarter of improved source samples contained fecal contamination in 39% of 191 studies; samples collected from rural areas and low-income countries were more likely to be contaminated as compared to samples from wealthier countries (Bain et al., 2014).

Diarrheal disease is the second leading cause of death in children under the age of 5 with 1.7 billion annual cases (WHO, 2017a) . Of the estimated 842,000 annual deaths within low- and middle-income countries (LMICs), 361,000 annual deaths are children under five years or nearly 1,000 child deaths a day. These deaths are due mainly to preventable diarrheal diseases as a result of unsafe drinking water, sanitation, and hand-hygiene, (WHO, 2018). However, a significant proportion of these cases are preventable through access to adequate quantities of safe water (WHO, 2017a). Illnesses related to inadequate water, sanitation and hygiene (WaSH) among children cause 443 million lost school days each year (Moszynski, 2006). Furthermore, according to other estimates, children under the age of 5 lose 6.8 billion healthy days to diarrheal illness each year due to lack of basic water supply and sanitation services (Hutton, Haller, & Bartram, 2007).

Access, availability, and safety must be taken into consideration when evaluating water services. The Joint Monitoring Program (JMP) created a service "ladder" for household drinking water services consisting of five water service ranks: 1) safely managed 2) basic 3) limited 4) unimproved and 5) surface waters.

Table 1.1 The five categories of household drinking water services as defined by JMP.

Service Level	Definition
Safely Managed	Drinking water from improved water sources and is located on premises; available when needed; free of fecal and priority contaminants
Basic	Drinking water from improved water sources; collection from source does not take more than 30 minutes, roundtrip to collect water (including queuing)
Limited	Drinking water from improved water sources; collection from source takes more than 30 minutes, roundtrip to collect water (including queuing)
Unimproved	Drinking water from unprotected dug wells or unprotected springs
Surface Waters (No Service)	Drinking water collected directly from rivers dams, lakes, streams, canals, or irrigation channels

Adapted from WHO and UNICEF (WHO & UNICEF, 2017b)

While 89% of the population has access to at least "basic" water services, 884 million people are only able to access limited and unimproved sources, of which 150 million people do not have access to any water services and rely on surface water sources for their drinking water needs (WHO & UNICEF, 2017b). Campaigns that focused on achieving the Millennium Development Goals, specifically to halve the global proportion of people without access to improved sources of water, have been successful; between 1990 and 2015, about 2.6 billion people gained access to improved drinking water sources (WHO & UNICEF, 2015). However, over 800 million people still do not have access to improved drinking water sources (WHO, 2014a). Furthermore, in LMICs, only 53% and 25% of urban and rural populations, respectively, have access to safely managed water services, posing a serious service gap that needs to be addressed.

Centralized water treatment, conventional piped water systems, and source water policies and regulations are common in high-income countries (HICs) and enable effective treatment and delivery of safe water to households. While larger cities within LMICs often have access to infrastructural resources, communities that experience urban sprawl and rural populations in LMICs may not have these water services in place. In 2015, an estimated 69% of the global population did not have access to piped water sources (WHO & UNICEF, 2017a). Although implementing conventional piped water systems and treatment facilities to deliver safe water to households are the ultimate goals of governments and public health agencies and the preferred means of providing safe water to all populations, many decades are needed to deploy long-term solutions in rural and developing parts of the world. This is because these types of infrastructural investments require technical expertise and large amounts of funding to build and maintain (Babu & Chaudhuri, 2005). Additionally, current water supplies in many LMICs still need further treatment prior to consumption because residual chlorine levels may not be high enough within the distribution system to achieve high quality drinking water (Reller, Mong, Hoekstra, & Quick, 2001; Weber et al., 2019). The responsibility of acquiring, treating, and storing water safely is essentially placed on many of the poorest communities (Sobsey, 2002). As utilities and governments improve service delivery, there is still a need for timely, achievable, and economical solutions to fill the gaps in these setting. Therefore, a focus on drinking water and treatment at the household level has been gaining attention (Thomas Clasen & Schmidt, 2007; Tom Clasen et al., 2015; Fewtrell et al., 2005).

In response to the service gaps, which can often result in microbial contamination and waterborne diseases, household water treatment systems (HWTSs), or point-of-use technologies (POUs), that are capable of reducing microbial and chemical contaminants are being adopted in

numerous communities among LMICs. Applied by individuals prior to consumption, these technologies are designed to treat water and/or prevent further contamination of stored water at the household level prior to consumption. HWTS and POU treatment technologies were developed to be utilized in low-resource settings, and, are therefore, intended to be practical and inexpensive to employ. These technologies aim to fill the current service gap in LMICs and improve health in these areas (Sobsey, 2006). A study that implemented two popular water filtration POU technologies among 88 households in a South African village saw a 96.2% reduction in diarrheal incidences (Charlotte Moropeng et al., 2018). Field trials implementing HWTSs and POUs as water treatment interventions have illustrated the ability of these technologies to reduce diarrheal disease among developing and rural populations (Clasen & Schmidt, 2007; Clasen et al., 2015). Further optimization of these technologies has the potential to provide increased protection against pathogenic contaminants.

CHAPTER 2: LITERATURE REVIEW

2.1 Household and Point-of-use Water Treatment Technologies

HWTSs and POUs are technologies that employ processes to physically, chemically, or biologically remove or destroy microbial and/or chemical contaminants in water. Although many POUs are simple to use, affordable, and practical for developing and rural communities, not all technologies deliver the same degree of water quality improvement (Agrawal & Bhalwar, 2011; Sobsey, 2002; Sobsey, Stauber, Casanova, Brown, & Elliott, 2008; WHO, 2011b). WHO has established quantitative performance targets for HWTSs by specifying the Log_{10} reduction benchmarks that must be met for bacterial, viral, and protozoan reductions (WHO, 2011b).

Table 2.1 WHO performance targets for bacterial, viral, and protozoan Log_{10}-reductions to meet specified levels of protection (WHO, 2014b).

★★★	: 4 log_{10} reductions of bacteria, 5 log_{10} reductions of viruses, and 4 log_{10} reductions of protozoa
★★	: 2 log_{10} reductions of bacteria, 3 log_{10} reductions of viruses, and 2 log_{10} reductions of protozoa
★	: Meets standards of at least two classes of pathogens for the 2-star benchmark

These microbial performance specifications are health-based targets and derived from quantitative microbial risk assessment (QMRA) methods (WHO, 2016). Three stars denotes comprehensive protection (very high pathogen reduction), two stars denotes comprehensive

protection (high pathogen reduction), and one star denotes targeted protection. A zero-star level indicates little or no protection.

Specific performance levels are associated with limiting the burden of disease, measured in Disability-Adjusted Life Years (DALYs). The protection from microbes delivered by a three-star level (highly protective) technology, assuming proper and consistent use, is associated with limiting lost DALYs attributable to water-related disease to below 10^{-6} per person. Two-star (protective) technologies are associated with limiting lost DALYs to below 10^{-4} per person (WHO, 2011a, 2016).

Membrane ultrafiltration technology falls in the three-star tier, while UV disinfection, chemical and solar disinfectants typically score in the two- and one-star tiers (WHO, 2016). Many POUs use a single barrier approach, utilizing a single treatment technology and microbial reduction mechanism to reduce microbial or chemical contaminants (i.e. filtration for removal or chemical disinfectants for deactivation, etc…). Although these technologies do achieve some reduction of contaminants, POUs do not consistently achieve protective (2-star) and highly protective (3-star) performance targets. Hence, there is a need for multi-barrier POU technologies that are able to improve the efficacy of drinking water treatment and increase the opportunities for pathogen removal or deactivation (WHO, 2016). Multi-barrier POUs are often simple and innovative in principle and employ treatment methods in the form of add-ons or combinations of existing technologies that offer a reliable and cost-effective option, complementing and improving single technologies. For example, the P&G Purifier of Water uses calcium hypochlorite as a disinfectant, ferric sulfate as a coagulating agent, and a fabric filter to facilitate removal of flocculated particles (WHO, 2016).

In Sobsey et al. (2008), popular POU technologies were documented for their effectiveness at removal of microbial contaminants in both laboratory and field settings. The POUs assessed in the study were ceramic water filters (CWFs), BioSand filters (BSF), solar disinfection (SODIS), free chlorine disinfection, and chemical coagulation with chlorination. The study found that Log_{10} Reduction Values (LRVs) achieved in field settings were less than half the LRVs achieved in a controlled laboratory setting, especially among human enteric viruses that are not reduced effectively by POU filtration technologies such as ceramic and BioSand microporous filters, indicating inadequate health protection in practice (**Table 2.2**) (Sobsey et al., 2008).

Table 2.2 Laboratory vs. field based studies of commonly used POU technologies

POU Technology	Pathogen	Field Results (LRV)	Laboratory Results (LRV)	Factors influencing performance efficacy
Ceramic Water Filters (CWF)	Bacteria Viruses Protozoa	2 0.5 4	6 4 6	Pore size, flow rate, filter composition, and metal augmentation (Sobsey, 2002; Brown et al., 2007; Brown, 2007)
Biosand filtration (BSF)	Bacteria Viruses Protozoa	1 0.5 2	3 3 5	Filter biological maturation, water dosing conditions, flow rates, water idle time within the sand layer, time between water dosing, granular media size, and challenge viral agent (Elliot et al., 2006 and 2008; Stauber et al., 2006; Palmateer et al., 1999)
Solar disinfection (SODIS)	Bacteria Viruses Protozoa	3 2 1	5.5+ 4+ 3+	Temperature, turbidity, water depth, oxygenation, light intensity, and exposure time (Sobsey, 2002; Wegelin et al., 1994; Reed, 1997; Kohn and Nelson, 2007; McGuigan et al., 2006)
Free chlorine disinfection	Bacteria Viruses Protozoa	3 3 3	6+ 6+ 5+	Chlorine concentration, contact time, and turbidity (Crittenden et al., 2005; Sobsey, 1989 and 2002)
Coagulation/ chlorination	Bacteria Viruses Protozoa	7 2-4.5 3	9 6 5	Turbidity and removal efficiency of chlorine resistant pathogens by coagulation (Souter et al., 2003; Sobsey, 2002)

Adapted from Sobsey et al., 2008.

While there is variation in observed LRVs, studies have noted reductions in adverse health effects, with all five of the listed POUs able to achieve 30-40% reduction in diarrheal disease (Thomas Clasen & Schmidt, 2007; Fewtrell et al., 2005).

The maximum LRVs may be contingent upon different factors, depending on the specific POU technology. For example, LRVs as a result of filtration through CWFs are influenced by pore size, flow rate, filter composition, and metal augmentation while LRVs achieved with the use of BSFs may rely more on filter biological maturation, water dosing conditions, flow rates, filter bed contact time, time between water dosing, granular media size, and challenge viral agent (J. Brown & Sobsey, 2009; Palmateer et al., 1999; Sobsey, 2002; Stauber et al., 2006). SODIS efficacy may be more dependent on total oxygen content, solar exposure time, temperature, turbidity and depth of water. Microbial reductions due to chlorination have been shown to be influenced by turbidity, chlorine demand, and chlorine contact time (Sobsey, 2002).

Simple cloth/saree filtration, specifically, has not been studied extensively for microbial reductions from drinking water. However, size of contaminant particles is known to be a key factor influencing LRVs because pore sizes of cloth filters are relatively large compared to other POU filtration technologies (Colwell et al., 2002; Huq et al., 1983). A research study using simple cloth filtration of surface water sources in Bangladesh observed that cholera bacterial particles have preferential attachment to copepods, small 1-5 mm aquatic organisms, that are large enough to be readily filtered out. Because the copepods were readily removed using the 4-8 layers of cloth filters, cholera bacteria particles were also partially removed, resulting in a 2-Log_{10} reduction in *V. cholerae*. This resulted in a 48% reduction in incidence of hospital cholera cases (Colwell et al., 2002)

2.2 Filtration as a POU

While reducing microbial contaminants can be accomplished through an array of methods, practicality of methods may vary among communities and users. For instance, boiling water is an effective way to inactivate microbial contaminants. Dry fire wood and charcoal are two of the many different fuel sources used to boil water. These fuel sources, however, can be expensive or in short supply among the communities that employ this practice. Firewood is also often damp and not readily flammable, especially in South Asia where seasonal monsoons last for months (Colwell et al., 2002). Chemical disinfectants are also effective at inactivating microbes but can be expensive and difficult to dose, and their performance may be influenced by water quality characteristics such as the presence of organic matter, reduced inorganic and organic nitrogen species, and/or reduced transition metals, which can influence chlorine demand. Chemical disinfectants also produce harmful disinfectant byproducts and fail to reduce turbidity (WHO, 2011a).

Traditional membrane technologies, including reverse osmosis filters, nanofilters, ultrafilters, microfilters, biofilters, and membrane bioreactors are effective at removal of microbial contaminants but are costly, difficult to maintain, and periodically require replacement. Therefore, traditional membrane technologies are not an attractive option as POUs in developing and rural communities (WHO, 2011a). Other filter-based POU treatment technologies have become more widespread and popular in application, especially among rural and developing populations. Granular media filters contain sand, diatomaceous earth, and other particulates organized in packed, yet porous, layers that retain microbes by physical removal, sedimentation, and adsorption. BioSand filters, a HWTS application of slow sand filtration, develop a biologically active layer that both physically removes and deactivates microbial contaminants from water sources (WHO, 2011b).

Ceramic water filters (CWFs) are a popular and effective type of POU technology. Similar to granular media filters and BioSand filters, CWFs are effective at removing microbial contaminants as well as reducing turbidity (Abebe, Chen, Sobsey, Gray, & Karanis, 2016). The filters are made of clay and combustible materials such as sawdust, rice, or coffee husks—producing a microporous filter matrix once fired in a kiln. Oftentimes, colloidal silver is added to the ceramic filters to enhance pathogen inactivation. As influent water passes through the CWFs, microbial and other colloidal particles that contribute to turbidity are filtered out, and clarified effluent is produced (Hoepfner, 2018). These filters decrease turbidity levels to as low as 0.2 NTU, meeting WHO standards of <1 NTU; municipal water treatment facilities are required to reduce turbidity to <0.3 NTU (Bartby, 2016). However, high viral removal with CWFs remains unsuccessful, achieving only 0.4 Log_{10} reductions and potentially failing to achieve the 1-star level of performance of health protection (Abebe et al., 2016; J. Brown & Sobsey, 2009; Joe Brown & Sobsey, 2010). While there is the potential to improve CWFs, high turbidity levels can limit their effectiveness by clogging the filter pores, potentially reducing the technology's life span and requiring constant cleaning by users (Mohamed et al., 2015).

2.3 Cloth Filtration

In addition to granular sand media, BioSand filters, and CWFs, cloth filtration, specifically saree cloth, is also a POU filtration method that is popular among developing and rural communities within South Asia. Saree cloth is a type of garment typically worn by South Asian women and is comprised of tightly woven threads, commonly made of cotton or silk. Due to the tightly woven threads, some contaminants are prevented from passing through, making the cloth a potential POU technology if properly optimized. The attraction of using cloth filtration as a POU stems from its household availability; scraps of available cloth can be used to filter water at the

household level. Although this HWTS method is widely used and accessible, cloth filtration has been only nominally effective.

A field study in rural parts of Bangladesh reported a 2 Log_{10} reduction in cholera bacterial particles by using 4-8 layers of saree cloth as a POU filtration technology. The 4-8 layers of saree cloth created a pore size of about 20 μm. When examining the relationship between *Vibrio cholerae* and copepods, small crustacean type organisms often found in surface water sources, it was reported that the bacteria has preferential attachment to copepod surfaces as well as egg cases and oral areas. The attachment of *V. cholerae* to copepods and the removal of the bacteria-laden copepods through cloth filtration by size exclusion superficially resembles the mechanism of bacterial removal through the use of coagulants prior to filtration (Huq et al., 1983). While the average virus with a 25-90 nm diameter may not be filtered out by cloth with a pore size of 20 μm, *V. cholerae*'s preferential attachment to copepods allows for bacterial particles to be readily filtered out. The study also noted that best results were achieved when using slightly worn saree cloth rather than new material; as the worn cloth frays to give agitated threads and fibers that contribute to the trapping and filtration of particles containing cholera bacteria (*Vibrio cholerae)* (Colwell et al., 2002).

A study using various layers of engineered fabric recorded 1.4 to 3.0 Log_{10} reductions of *E. coli* as well as an exponential decrease in turbidity with the addition of fabric layers; however, viruses were not studied (Siwila & Brink, 2019). Viruses and other bacteria are too small to be size-excluded by cloth filtration. However, if microorganisms are or become particle-associated, achieving higher LRVs may be possible.

2.4 Coagulation

The process of coagulation-flocculation and sedimentation occurs when positively charged chemical compounds, known as coagulants, are added to water and bind with negatively charged particles, including clay, natural organic matter, microorganisms and other colloidal particles. The binding of coagulants and negatively charged contaminants creates flocs that settle out under the influence of gravity (Bartby, 2016). Coagulation describes the chemical process by which particles collide and remain together, whereas flocculation describes the mechanical process by which coagulated particles form and grow into larger floc particles. Generally, the greater the charge, size, and molecular weight of the coagulant, the more effective the compound is at coagulating contaminants and improving water quality (Shammas, 2005).The two main chemical coagulation mechanisms regarding synthetic polymeric and chemical coagulants are 1) charge neutralization and 2) inter-particle bridging (Shammas, 2005).

Charge neutralization occurs when the positive charge of a coagulant in solution interacts with the opposite charge of various microorganisms or particulates. Many contaminant particles, such as silt, clay, viruses, and bacteria, have negatively charged surfaces, and positively charged coagulants are able to react effectively, resulting in a near net-zero total surface charge. Particle bridging occurs when polymeric coagulants combine with colloidal particles to form large, polymeric, colloidal structures. Polymeric coagulants can branch out and adhere to multiple colloids as a result of the many available points of attachment on the compound, which allows for bridging among particulates and other formed floc structures, further facilitating floc formation (Shammas, 2005). Inter-particle bridging can be broken down into four stages: 1) dispersion, 2) adsorption, 3) compression and settling, and 4) collision (Bartby, 2016) Once the coagulant adsorbs to the surface of multiple colloids, forming multiple, larger flocs, the structure can precipitate out, contributing to the sedimentation stage (Bartby, 2016).

Coagulation has been used throughout history as a means of improving water quality. The Egyptians and Romans employed the process of alum coagulation as early as 1500B.C. and 77 A.D., respectively, and the English used alum as a coagulant in municipal water treatment in England as early as 1757 (IWA, 2019). Today, conventional water treatment plants typically employ four main processes when treating influent water: 1) coagulation-flocculation 2) sedimentation 3) filtration and 4) disinfection (Bartby, 2016).

Reducing turbidity levels is crucial to improve drinking water quality. Particulate matter such as clay, silt, inorganic and organic matter, and algae contribute to turbidity and can provide pathogens with nutrients and surfaces to adhere to and grown on. These turbidity causing particles can also contribute to pathogenic regrowth and aid in microbial survival by providing "shelter" from disinfectants. These particle-associated pathogens, if consumed, can cause waterborne diseases. While turbidity is not used as a health risk indicator, studies have suggested a strong relationship between turbidity and protozoan removal (USGS, 2019) and the World Health Organization recommends reducing turbidity of drinking water for potential health protection (WHO, 2017b). Benchmarks for turbidity of treated water have dropped from the 1989 standard of 1.0 NTU to current standards of 0.3 NTU; however, to protect against pathogenic contaminants, many water utilities have opted to produce effluent with turbidity levels ≤ 0.1 NTU.

Coagulation-flocculation steps are standardized and considered critical stages in water treatment processes that can effectively reduce turbidity, organic material, microorganisms, and some metals, contributing to water clarification. Additionally, the resulting decrease in suspended particulates and organics also improves disinfection performance which further improves the quality of effluent waters. For these reasons, coagulation-flocculation is considered one of the most important steps in the physical removal of contaminants by many treatment facilities (Bartby,

2016; Bellamy, Cleasby, Logsdon, & Allen, 1993; Cleasby, 1989; Matilainen, Vepsäläinen, & Sillanpää, 2010; WHO, 2017b).

Inorganic coagulants are more commonly used than organic coagulants in municipal water treatment facilities. These coagulants are generally categorized into those that are aluminum based (aluminum sulfate, aluminum chloride, and sodium aluminate) and those that are iron based (ferric sulfate, ferrous sulfate, ferric chloride, and ferric chloride sulfate). Aluminum and iron based coagulants work by forming a charged poly-nuclear complex; the ions then quickly hydrolyze (Bartby, 2016). Hydrated lime and magnesium carbonate are also commonly used as coagulants, but more so for hard water. Mixing speeds, pH of influent water, and coagulant dosing are all important factors to consider when determining which chemical coagulant hydrolysis species is effective.

While coagulation-flocculation and filtration alone are two possible POU technologies, using the two as a multi-barrier technology has the potential to be more effective at removing pathogenic contaminants (Abebe et al., 2016).

The Procter & Gamble's PUR packet is one such product that utilizes a multi-barrier approach; the PUR packet contains calcium hypochlorite for disinfection and ferric sulfate, an inorganic coagulant. Users are instructed to pour the packet's contents into 10 liters of water, rapidly mix for five minutes, let the water stand until observable settling of the floc is complete, pour the water through a cloth, and let the water stand for 20 minutes for further disinfection. Laboratory studies of the PUR packet plus cloth filtration, using both EPA-model and field-sample water from developing countries with spiked microbes, resulted in $\geq$ 4-Log_{10} reductions of polio and rotavirus and $\geq$3-Log_{10} in *Cryptosporidium parvum and Giardia lamblia* (Souter et al., 2003). A HWTS field study in Tanzania with 390 households looked at Log_{10} reductions across six

different technologies. One technology, the PUR packet followed by filtration, resulted in ≥ 2.9-Log_{10} reductions in thermotolerant coliforms (Mohamed et al., 2015). The PUR packets have shown that a multi-barrier approach to POU technologies can be more effective than single-barrier approaches (WHO, 2016).

Although effective at reducing turbidity and promoting the settling out particulate matter and microorganisms, inorganic coagulant performance is highly sensitive to changes in pH, temperature, and ionic strength and must be appropriately and accurately dosed to achieve maximum microbial and particulate matter removal. Specialized instruments and sensors are available in municipal water and wastewater treatment facilities to characterize influent water sources, calculate optimal dosing, and monitor treatment to ultimately ensure effective treatment. With regards to POU applications, these critical process control steps are not feasible, making proper dosing extremely challenging, given the potential for highly variable influent water quality conditions. Furthermore, inorganic coagulants produce harmful sludge byproducts that can be difficult to safely dispose of and can accumulate in the environment. Studies have reported that floc solid residuals from inorganic salts and other aluminum-based inorganic coagulants pose health risks, and the lifetime cumulative intake has been shown to play a role in the development of Alzheimer's disease and other neurological disorders (Liu, Li, Zhang, Nie, & Wang, 2013; Matilainen et al., 2010). Inorganic coagulants effectively achieve high microbial reductions, but their need for precise dosing, performance variability with dose, and potential toxicity make inorganic coagulants an unattractive choice for POUs where there is a need for technologies that are safe, simple, and practical.

Plant based, organic coagulants, such as those from *Morgina oleifera* and *Strychnos potatorum,* have been used in rural communities for centuries. *M. oleifera,* a flowering tree found

in South Asia and West Africa, has been referred to in many religious Hindu scriptures, such as the *Vedas* (Imran Ali et al., 2011). The *Moringa oleifera* plant has been observed to have seeds with coagulant properties and has been used for water clarification techniques for generations in rural Indian communities (Imran Ali et al., 2011). A study in rural and low-income Indian communities explored the effectiveness of *Moringa oleifera* and saree-cloth filtration as a pre-treatment step prior to UV disinfection as a point-of-use water treatment system. The study used surface water samples from peri-urban slums in Chennai, India and assessed the effluent waters for turbidity, organic content by chemical oxygen demand (COD), and coliform bacteria concentration as MPN values. Laboratory studies have achieved 1-2 Log_{10} bacterial reductions (Madsen, Schlundt, Fadil, & Omerf, 1987; Nkurunziza, Nduwayezu, Banadda, & Nhapi, 2009). However, various other studies have also documented secondary bacterial re-growth 24 hours after the initial *M. oleifera* treatment (Madsen et al., 1987; Oluduro & Aderiye, 2007). While the plant-based coagulant did show removal of seeded *E. coli* in challenge waters in a laboratory setting, *M. oeifera* was not able to effectively remove coliform from stored village water (Firth et al., 2010). The effectiveness of *S. potatorum* and *M. oleifera* to achieve Log_{10}-reductions in conjunction with filtration was also studied, and ~2-Log_{10} reductions for bacteria and ~3-Log_{10} reductions for viruses were achieved. While these plant species do have some coagulating effects, there are concerns for bacterial regrowth in treated water with the use of *M. oleifera*. Furthermore, plant species that can be used as coagulants are not always abundantly and readily available (Babu & Chaudhuri, 2005).

2.5 Chitosan

Chitosan is an organic, biodegradable linear polysaccharide of repeating N-acetyl-D-glucosamine and D-glucosamine monomers that is derived from the chemical treatment of chitin

(**Figure 2.1**). Chitin is the second most abundant polysaccharide in the world and is a primary component of the exoskeletons of crustaceans. Chitosan is produced by chemically de-acetylating chitin using an alkaline treatment to form modified polymers (**Figure 2.1**).

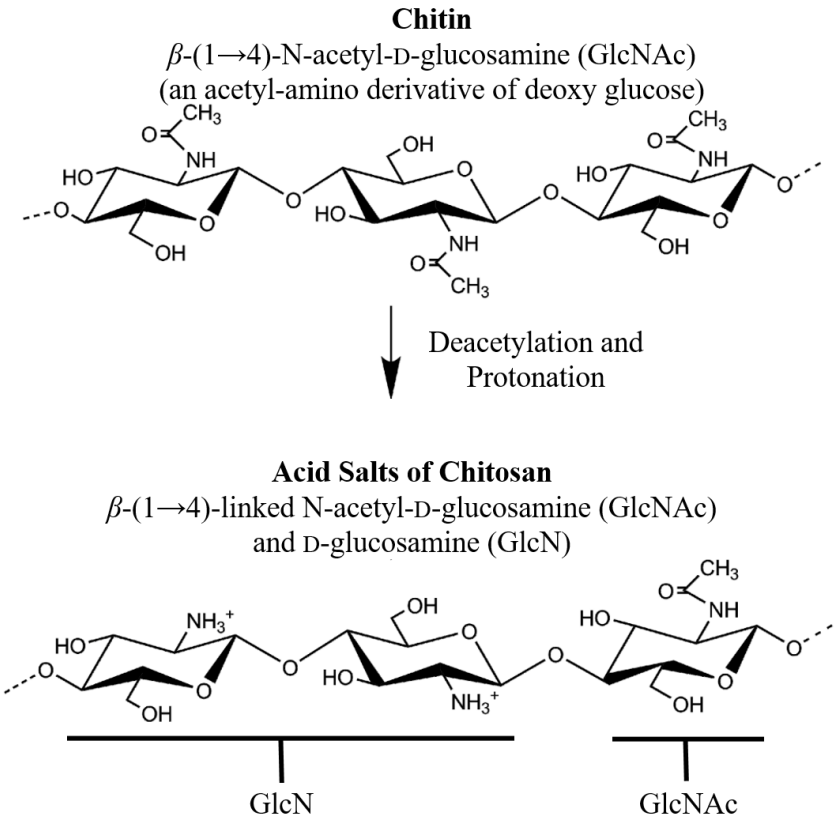

Figure 2.1 Molecular structures of chitin and chitosan

While chitosan does not readily dissolve in water, acid salts of chitosan do and are easy to chemically derive from chitin. An alkaline treatment deacetylates chitin, exposing amino groups ($-NH_2$). The amino group has a pK_a value of ~6.5, which leads to protonation in neutral waters that increases with acidity. Chitin then becomes soluble in acidic aqueous media, forming chitosan that is readily water soluble. The protonated amino groups give chitosan its positive charge, a critical property of effective coagulants. A higher positive charge density in water engenders effective removal of microbes and particulate matter that cause turbidity (Rinaudo, 2006). Because deacetylation allows for the protonation of exposed amino groups, a higher degree of deacetylation

(DD), ranging from 40% to 98%, is also associated with higher solubility and a higher number of positive charges along the polymeric chain (M. N. V. Ravi Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004).

When added to water, chitosan acts as a coagulant to facilitate the physical removal, by filtration, of viruses, bacteria, and other colloidal particulate matter that is captured onto larger flocs that settle out (Abebe et al., 2016).

The ability of chitosan salts to remove microbial contaminants from water through the coagulation-flocculation and sedimentation procedure is not well documented. Studies have observed an inverse relationship with chitosan DD and required chitosan dose; the higher the DD, the lower the required dose. For example, a DD of 99%, as compared to a DD of 38%, required 10x lower dose of chitosan hydrochloride (Sabina P Strand, Vandvik, Vårum, & Østgaard, 2001).

In terms of safety, chitosan is non-toxic and has been used in a variety of foods, nutritional supplements, cosmetic products, drug therapies, and other medical applications (Majeti N V Ravi Kumar, 2000).

In addition to being biodegradable, non-toxic, and abundant, chitosan pre-treatment is also able to achieve microbial reductions without being significantly affected by pH or coagulant dosing. These unique characteristics make chitosan a strong candidate for use in water treatment, specifically with POUs (Abebe et al., 2016). The effects of coagulation-flocculation and sedimentation procedures and conditions on chitosan floc formation and floc particle size distribution still need to be further explored.

2.6 Filtration and Chitosan

A lab study employing buffer test waters, jar test methods, and various doses of 3-10 mg/L of various chitosan types yielded 3-5 Log_{10} reductions for *E. coli* and bacteriophage MS2 (Soros,

Sobsey, Casanova, Ball, & Stewart, 2015). Another study evaluated the bacterial, viral, and turbidity reductions achieved from the use of water-soluble chitosans followed by filtration through CWFs (Abebe et al., 2016). Chemically-defined test waters supplemented with kaolinite clay, *E. coli* KO11 bacteria, and MS2 coliphage were pre-treated with doses ranging from 5-30 mg/L using three specific chitosan salt types: chitosan hydrochloride (HCl), chitosan acetate (CH_3COO^-), and chitosan lactate ($CH_3CH(OH)CO_2^-$). After the coagulation-flocculation and sedimentation procedures, the supernatant water was poured into the CWFs. The combined effects of the chitosan pre-treatment and physical removal by the CWFs, resulted in mean 4-7.5 Log_{10} reductions for *E. coli* KO11 and mean 3-4.5 Log_{10} reductions for MS2 coliphages, with chitosan acetate yielding the greatest reduction of the three tested coagulants. Turbidity was also consistently reduced to <1 NTU meeting the turbidity standards set by US EPA and WHO. Based on the WHO HWTS performance targets, the combined efforts of chitosan pre-treatment and CWF filtration met the 2-star level performance of health protection (Abebe et al., 2016).

While many viruses and some other pathogens are too small to be sufficiently filtered out by microporous filters alone, the use of chitosan as a pre-treatment step bas been shown to increase microbial reduction when combined with filtration (Abebe et al., 2016). While use of cloth filtration, in its current state, does remove particle-associated bacteria by size-exclusion to varying degrees, other microorganisms would easily pass through due to their small size. As cloth filtration is already a common practice among many developing and rural communities in South Asia, a chemical coagulation pre-treatment step prior to filtration, could coagulate and flocculate bacteria and viruses to greatly improve microbial and turbidity reductions (Colwell et al., 2002).

A better understanding and optimization of chitosan floc size is crucial to maximizing its ability to function as an effective coagulant, especially if it is used in conjunction with POU

technologies such as cloth filtration, where the particle size of contaminants being filtered out is critical for maximizing microbial LRVs.

2.7 Particle Size Measurement Methods

The removal of suspended particles after coagulation-flocculation pre-treatment is vital to water treatment in both POU technologies and municipal water-treatment. Contaminant removal performance greatly depends on the size of these suspended particles; however, monitoring and capturing the true size, formation, and transformation of flocs in suspended water has proven to be difficult, but not impossible. Careful quantitative research concerning size distributions and concentrations of flocs in treated water is still lacking. Because flocs are porous and highly irregular, measurements of particle size are not always as accurate as the measurement of sediments, or more solid and regularly shaped particles (Xu, Fitzpatrick, & Gregory, 2008).

Laser and light scattering methods such as *static light scattering* (SLS) and *dynamic light scattering* (DLS) are often used to measure and identify particles that are several micrometers in size. Variations in scattered light are measured and used to calculate the size of the particle. Granulometers use the low-angle light scattering technique. This technique allows for the measurement of scattered light intensity, which is a function of the wave vector, or the difference between the falling and scattered ray (**Figure2.2)**. One study analyzed particle size and fractal dimensions of a suspension that contained raw, treated, and activated sludge using a laser granulometer and was able to measure suspended particle within samples (Kusnierz & Wiercik, 2016).

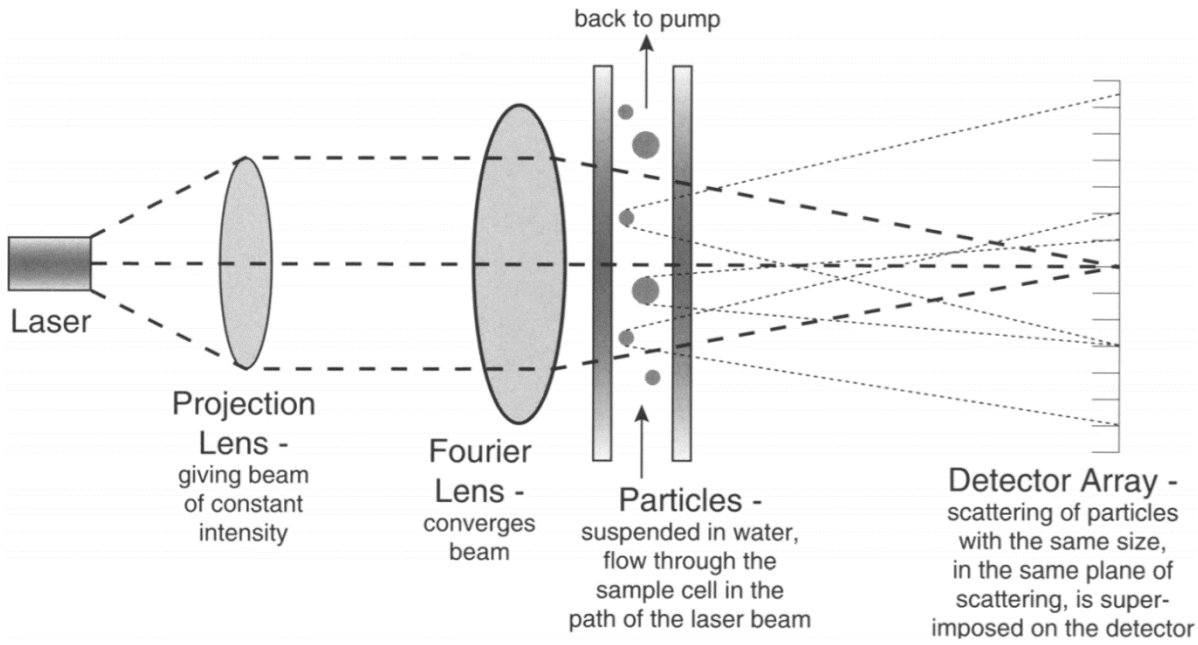

Figure 2.2 Schematic of a granulometer illustrating particles scattering light (Blott, Croft, Pye, Saye, & Wilson, 2004)

Coulter counters have accomplished particle size characterization. However, this method usually requires physical removal of a sample which could disturb formed flocs. Photography and videography analysis has been used to observe floc suspension and formation (Sun, Weber-Shirk, & Lion, 2016). Photometric dispersion analyzers (PDA) have also been used to measure floc sizes. This technique uses a light beam that passes through a flowing suspension, and the light intensity and the root mean square value of the fluctuating particles are then measured. PDAs are an effective option for showing qualitative changes in floc formation and aggregation (Ramphal & Sibiya, 2014).

Particle size analyzers (PSA) are another type of a measurement instrument that relies on a constant flow of suspended particles. PSAs use a laser beam that passes through the flowing suspension; particles scatter the light which is then used to approximate size (Xu et al., 2008).

2.8 Mastersizer 3000 by Malvern

The Mastersizer 3000 by Malvern is a PSA instrument that has the ability to measure particles that range from 0.01 µm – 3,500 µm. The instrument uses laser diffraction measurements which are collected when a laser beam passes through dispersed particulates. The scattered light's angular variation and intensity are measured and are then analyzed to calculate the size of particles that pass through the measurement cell that created the scattering pattern. As illustrated in **Figure 2.3**, smaller particles scatter light at a larger angle, and larger particles scatter light at a smaller angle. The Mie theory of light scattering is used to make these calculations and reports the size as a volume equivalent sphere diameter. The Mie theory's assumption that the particles measured are perfect spheres is a modeling simplification (Malvern, 2013). To achieve one unique number to describe a non-spherical particle, different features can be compared between the irregular particle to be measured and a theoretical spherical particle:

1) *equivalent surface area*—finding a diameter of a sphere that has the same surface area as the measured irregular particle

2) *equivalent maximum length*—finding a sphere with a diameter equivalent to the maximum length of the irregular particle

3) *equivalent minimum length*—finding a sphere with a diameter equivalent to the minimum length of the irregular particle

4) *equivalent volumes*—finding a sphere with a volume equivalent to the volume of the irregular particle

These features describe the technique of measuring irregular particles called "equivalent spheres". The Mastersizer 3000 by Malvern utilizes the fourth technique listed, equivalent volumes (Malvern, 2013).

Red and blue light sources, as illustrated in **Figure 2.4**, are used to measure across the entire 10 nm - 3.5 mm size range. The red light aids in the measurement of larger particles while the blue light aids in the measurement of smaller particles.

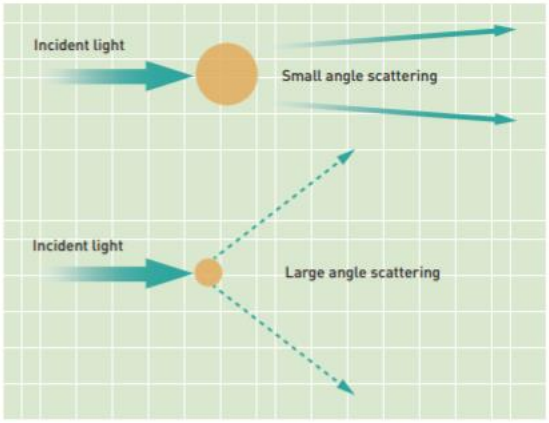

Figure 2.3 illustrates the how scattering angles differ among larger and smaller particles that pass through the Mastersizer measuring cell. (image from Malvern).

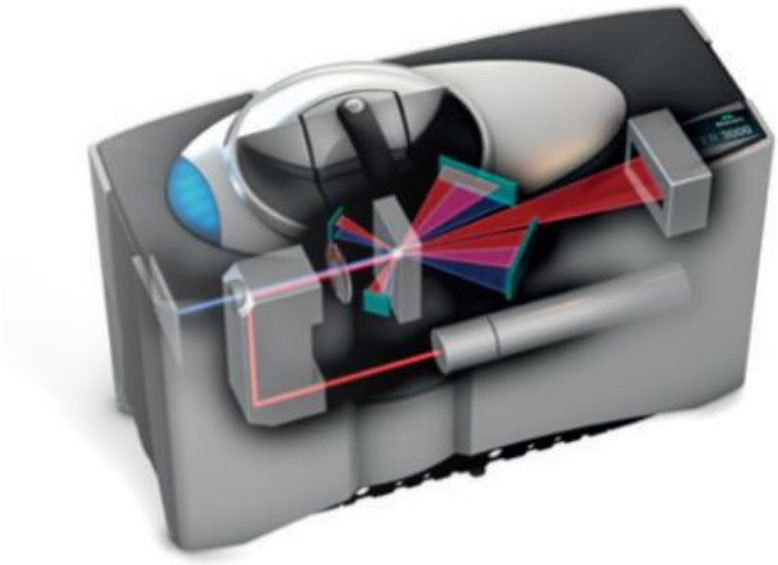

Figure 2.4 illustrates the Mastersizer 3000 by Malvern and the red and blue laser beam sources that aid in the detection of larger and smaller particles (image from Malvern).

A study evaluating the sizes of particles formed during municipal wastewater treatment aimed to measure floc sizes. While the study measured floc sizes in wastewater samples, the study

was able to successfully use the Mastersizer 3000 to analyze aggregate floc sizes formed during chemical coagulation (Lech, Marta, Michal, Harsha, & Krystyna, 2017).

The Mastersizer 3000 by Malvern was chosen to characterize and quantify the floc particle sizes and concentrations two types of test waters treated with 10 mg/L of chitosan acetate and three different stirring conditions during the flocculation process prior to cloth filtration. While other instruments such as a PDA, granulometer, or Coulter counter may have also been an attractive choice, the Mastersizer 3000 PSA instrument was readily available.

2.9 Objective

While studies have shown the effectiveness of chitosan as a pre-treatment for POU water treatment technologies, there is still much to be learned about the use of this organic coagulant. Specific dosing for partnered filtration devices, optimal coagulation-flocculation and sedimentation procedures, and floc size distributions and characterizations are all unknown factors of chitosan coagulation-flocculation, preceding filtration.

The goal of this project was to 1) quantify *E. coli* KO11, MS2 coliphage, and turbidity reductions in seeded test water of defined quality, that has been subjected to chitosan coagulation under different flocculation stirring conditions, followed by layers of cloth filtration, and 2) analyze floc particle sizes formed during coagulation and flocculation in waters of different quality and with different flocculation stirring conditions.

Table 2.3 Parameter conditions for microbial experiments

Filtration after Chitosan Pre-treatment	Test Microorganisms	Challenge Water (August collection)	Chitosan Acetate Dose	Stirring conditions	Replicates
12-layers of 100% cotton cloth	*E. coli* KO11 MS2 coliphage	Natural Lake Water	0 mg/L	NA	x3
			10 mg/L	Standard	
				Intermediate	
				Minimal	
		Natural Lake Water +1% Pasteurized Sewage	0 mg/L	NA	
			10 mg/L	Standard	
				Intermediate	
				Minimal	

Table 2.4 Parameter conditions for particle size analysis experiments

Chitosan Acetate Dose	Challenge Water	Stirring conditions *(triplicate samples)*	Replicates
10 mg/L	Natural Lake Water (August 18, 2018)	Standard	x3
		Intermediate	
		Minimal	
	Natural Lake Water + 1% Pasteurized Sewage (March 1, 2019)	Standard	
		Intermediate	
		Minimal	

CHAPTER 3: METHODS

3.1 Chitosan

Chitosan acetate (CH_3COO^-) (Food Grade Chitosan purchased from *Sarchem Laboratories, Inc.* in powder form) was chosen for use in this experiment. This type of modified chitosan was selected based on the results of previous studies comparing chitosan hydrochloride, chitosan acetate, and chitosan lactate. In that study, the use of chitosan acetate resulted in higher LRVs, ranging from 2.8-4.5 Log_{10}-reduction in MS2 *coliphage*, compared to other acid-modified chitosans (Abebe et al., 2016). The DD is 90.3% and the pH is 4.2 (**Appendix C**). The full chitosan analysis certificate from *Sarchem Laboratories Inc.* is in **Appendix C.**

3.2 Cloth Filtration

A 100% cotton linen material (200-count), bought from the bedding section of Wal-Mart, was layered twelve times and used as a filtration device. Twelve layers of the material was chosen based on preliminary experiments from 2017, which evaluated various layers and cloth types, to maximize the removal of test microbes from chitosan coagulated and flocculated water. Each layer was a square piece of 100% cotton linen material; 12 layers of the square material were stacked to create a filter.

3.3 Overview of experimental evaluation methods

A 1% bleach solution, diluted with deionized water from an 87,000 mg/L solution of available sodium hypochlorite, produced an 870 mg/L solution of available sodium hypochlorite. The cloth filters were soaked in this 1% bleach solution for 30 minutes, rinsed with deionized

water, and air dried for 24 hours. The filter effluent water collection containers were also soaked in a bleach solution for 30 minutes and rinsed with deionized water before being scrubbed and washed with soap and finally rinsed again with deionized water. Each container was sterilized with a 70% ethanol solution. After presoaking the cloth filters in deionized water, they were secured to a PVC column by tightly wrapping four to five rubber bands around the column (**Figure 3.1**). This cloth filter set-up ensured a tight seal that prevented unfiltered influent water from entering the effluent receiving container. The filter-column apparatus was placed over a 2 L beaker used to collect the filtrate.

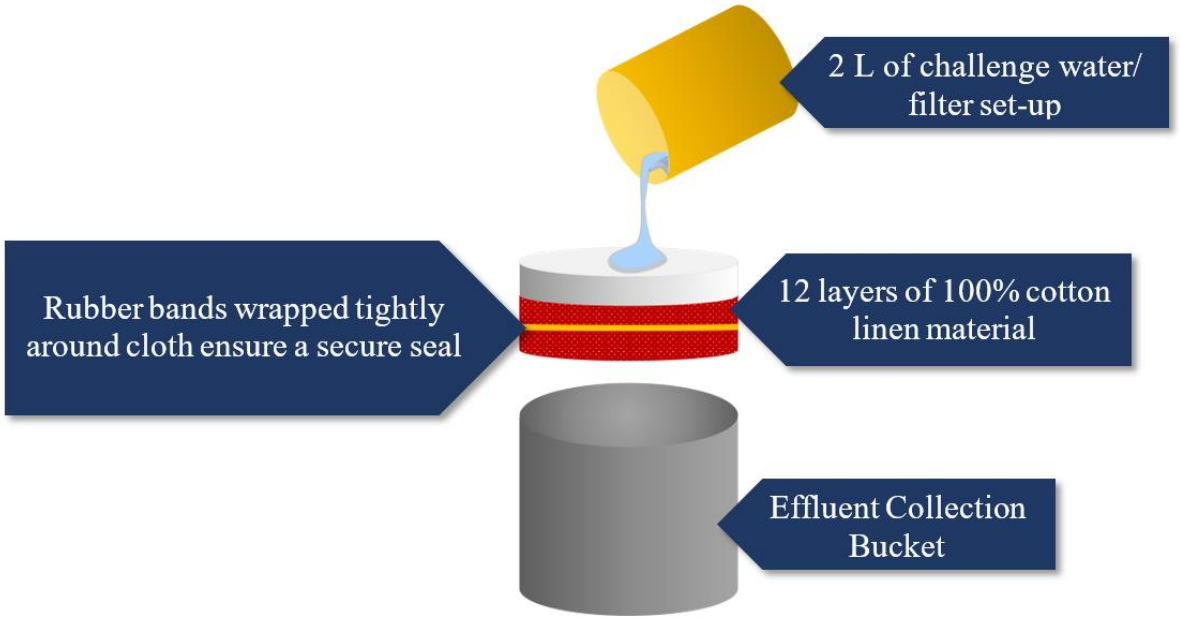

Figure 3.1 illustrates and details the cloth filtration apparatus.

3.4 Water Source

Natural lake water was obtained from University Lake on August 18, 2018 and March 1, 2019 and stored at 4°C. Water quality parameters can be found in **Table 4.1**. The natural waters were spiked with *E. coli* KO11 (ATCC #55124) and Male-specific (F+) coliphage (bacteriophage)

MS2 (ATCC #15597-B1) each with a concentration $\geq 10^6$ organisms per 100 mL to create influent challenge waters. These test water conditions provided a microbial concentration great enough to measure at most a 6-Log_{10} reduction, if achieved.

3.5 Challenge Waters

Two challenge waters were used: 1) natural lake water and 2) natural lake water amended with 1% by volume pasteurized sewage. The lake water was collected from University Lake in Chapel Hill, N.C. and primary effluent sewage was collected from the Orange Water and Sewer Authority (OWASA) Mason Farm Wastewater Treatment Plant.

Escherichia coli KO11 was the bacterium used because *E. co*li is an indicator for pathogenic enteric bacteria. MS2, a male-specific coliphage, was used as the model virus because its molecular characteristics, composition, and physical properties are similar to that of other human enteric viruses of health concern, such as noroviruses, enteroviruses, and astroviruses. Removal of MS2 coliphage is expected to be similar to removal of other human enteric viruses (WHO, 2016). *Escherichia coli* strain F_{amp} was used as the host for propagation and infectivity assay of MS2 coliphage.

A ~200 µL scrape of frozen *E. coli* KO11 culture of 10^9 colony forming units (CFU)/ 1 mL was dispensed into a flask of 50 mL of tryptic soy broth (TSB). A 0.5 mL volume of chloramphenicol antibiotic 100x stock was added to the *E. coli* KO11 overnight culture broth prior to incubation to impede the growth of other competing organisms in order to produce a pure culture of *E. coli* KO11. The 100x stock concentration of chloramphenicol was prepared by filtering a 3.4 g/L of chloramphenicol in 100% ethanol solution though a 0.22-µm-pore-size membrane filter. The suspension of *E. coli* in TSB was then incubated on a shaker (at 100 rpm) for 24 hours at 37°C to create the overnight bacteria suspension to then purify and seed into test water. After the

incubation period, the culture was centrifuged at 3000x gravity for 10 minutes at 4 ^{o}C, and the sedimented cells were washed again with phosphate buffer for three successive rounds. The phosphate buffer was made by autoclaving and cooling a solution of 0.125% by volume of phosphate stock buffer (34.0 g of KH_2PO_4 in 500 mL distilled water, adjusted to a pH of 7.2 with a 1 M NaOH solution, and then diluted to 1 L with distilled water), 0.5% by volume of 0.4 M $MgCl_2$, and deionized water. Approximately 20 mL of the washed *E. coli* KO11 suspension were added per 10 liters of challenge water, producing a concentration of ~10^6 CFU/mL of *E. coli* KO11.

An entire 1 mL frozen stock sample of MS2 coliphage, with a titer of 1x10^{11} plaque forming units (PFU) per milliliter, was thawed and spiked into the challenge waters as well. This produced a 10^6-10^8 PFU/mL suspension of MS2. The 1% by volume, pasteurized sewage challenge water was prepared by using the same microbial methods with the addition of raw sewage (collected from OWASA each time per experimental day) that had been pasteurized at 70 ^{o}C for 30 minutes in a water bath. For every 2 L of challenge water, 20 mL of pasteurized primary effluent sewage was added. The pasteurized sewage included the settled and suspended particles. Each filter was dosed with 2 L of a challenge water pre-treated with 10 mg/L of chitosan acetate followed by one of three stirring conditions or challenge water that was not pre-treated with chitosan. The chitosan acetate dose of 10 mg/L was chosen based on preliminary experiments (**Appendix A**).

3.6 Chitosan Treatment and Filtration

Prior to treatment with chitosan, a sample of influent water (containing test microbes and pasteurized sewage for certain experiments) was taken for microbial, physical, and chemical analysis. Two liters of influent challenge waters were prepared for each filter and placed in separate containers. To make a chitosan concentration of 10 mg/L per two liters of challenge water, a 10.0

mL volume of 2 g/L of chitosan acetate stock solution was measured and added to each 2 L of challenge water. Directly after the addition of chitosan, the water was mixed using a jar-test-flocculator apparatus containing paddle blade stirrers to initiate the coagulation-flocculation processes. Water was stirred initially at 100 RPM for 1 minute and thereafter according to the conditions detailed in **Table 3.2** for three different stirring conditions. After the allotted flocculation time, a sample of the post-flocculation treatment influent water was taken from the middle of the influent bucket and one inch below the surface. The collected sample was then used for microbial, physical, and chemical testing. The supernatant water of the two liters of challenge water were passed through the cloth filter apparatus, leaving 100 mL of settled flocs. A sample of each cloth filter effluent was then taken for microbial, physical, and chemical testing.

Each experiment was run in triplicate. Concentrations of *E. coli* bacteria, MS2 coliphages and turbidity were determined in each sample and then $\log_{10}$ microbial reductions were calculated based on the differences in $\log_{10}$ concentrations among influent waters, cloth filter effluent (untreated) waters, post-treated (coagulated-flocculated-settled) waters, and cloth filter effluent (treated) waters.

Table 3.1 Experimental parameters that included two different challenge waters, 0 and 10 mg/L concentrations of chitosan acetate, and three different floc stirring conditions.

Challenge Water	Chitosan Acetate Dose	Stirring conditions *(triplicate samples)*
Natural Lake Water	0 mg/L	NA
	10 mg/L	Standard
		Intermediate
		Minimal
Natural Lake Water + 1% Pasteurized Sewage	0 mg/L	NA
	10 mg/L	Standard
		Intermediate
		Minimal

Table 3.2 The three stirring conditions used, standard, intermediate, and minimal, were defined by various mixing and settling conditions.

Stirring Condition	Duration (minutes)	Mixing Speed (RPM)
Standard	1	100
	15	25
	30	0
Intermediate	1	100
	2	25
	5	0
	2	25
	5	0
	2	25
	30	0
Minimal	1	100
	30	0

The three different stirring conditions were chosen to resemble three different levels of mixing. The standard conditions are standard coagulation-flocculation and sedimentation procedures that are robust but not as practical in a field setting. Minimal conditions may be more practical in a field setting but may not facilitate proper floc formation, with just one minute of rapid stirring. An intermediate stirring condition was defined with the aim to create a mixing procedure that facilitated floc formation while still having the potential to be practical in a field setting.

3.7 Filter Cloth Decontamination

Cloth filters were reused for experiments and decontaminated between uses. A 1% bleach solution from an 87,000 mg/L solution of available sodium hypochlorite was prepared, resulting in an 870 mg/L solution of available sodium hypochlorite. After cloth filtration was complete and all samples of test water were properly collected, each cloth filter was soaked in this 1% bleach solution for 30 minutes**,** rinsed with deionized water, and then air dried for 24 hours.

3.8 Microbial Sampling Points

Log_{10} concentrations of *E. coli* KO11 and coliphage MS2 were determined. Log_{10} reductions were calculated from differences in concentrations for the numbered sampling points listed in **Figure 3.2** to assess removal due to the following processes: filtration alone, coagulation-flocculation-sedimentation pre-treatment alone, isolated effects of filtration after pre-treatment, and pre-treatment and filtration together.

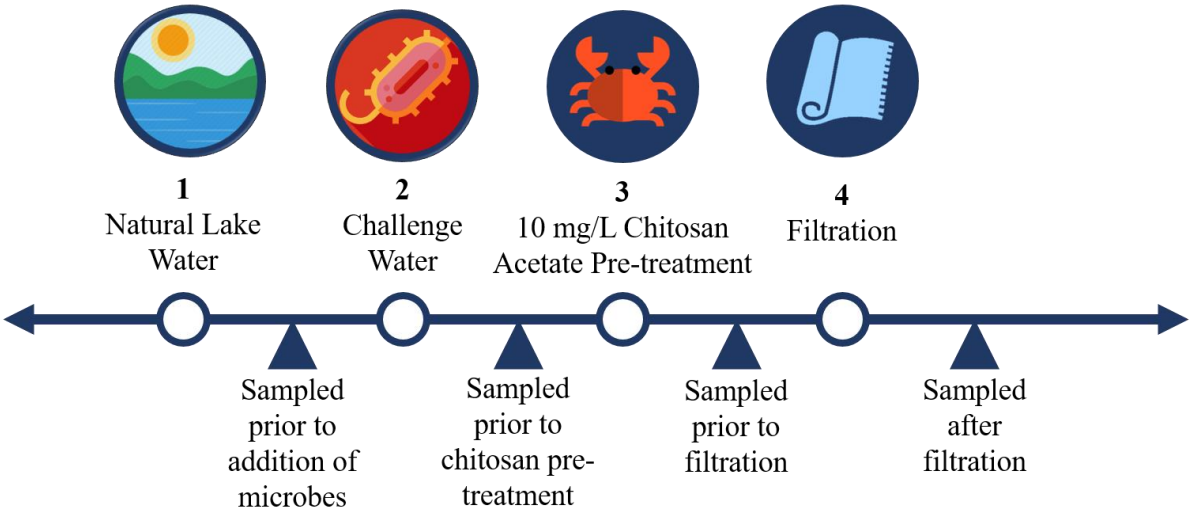

Figure 3.2 Schematic of water sampling points throughout experimental run.

3.9 Microbiological Methods for Bacteria Enumeration in Experiments

Dilutions were made of test water influent, filtered-only effluent, post-chitosan coagulation-flocculation-sedimentation treated influent, and post-filtration effluent for both chitosan concentrations. The enumeration of the *E. coli* KO11 for influent and effluent samples was done by spread plating the samples onto a 100 x 15mm Petri dishes of 1x Awesome Agar (AA). A mL amount (1% by volume) of chloramphenicol 100x stock solution (3.4 g/L chloramphenicol) was added per 500 mL of molten AA media after autoclaving and cooling of AA plates used to culture *E. coli* KO11. Chloramphenicol was added to AA medium to prevent the growth of other organisms. A 100 µL sample of test water was spread onto each plate. Samples

were plated in duplicates. The plates were then inverted and incubated for 18-24 hours at 37°C and after the incubation period, the *E. coli* colonies on each plate were observed, counted, and recorded.

3.10 Microbiological Methods for Virus Enumeration in Experiments

For the influent and effluent samples, double agar layer plaque assays were used as described in US EPA method 1601 (EPA, 2001). The bottom agar medium of 1.5x TSA was prepared by adding 60 g of TSA per 1 L of deionized water. After autoclaving, the agar was cooled to 50°C in the water bath and 10 mL of Strep/Amp 100x antibiotic stock concentrate (containing 1.5 g/mL streptomycin sulfate and 1.5 g/mL ampicillin sodium salt, dissolved in deionized water, and filtered through a 0.22-μm-pore-size membrane filter) and 2.5 mL of 4 M $MgCl_2$ were added per liter of 1.5x TSA medium. Then, 12-15 mL of molten agar was plated into 100 x 15mm Petri dishes. The top 0.7x TSA molten agar medium was prepared by autoclaving a mixture of 28 g of TSA dehydrated medium per liter of deionized water. After the mixture was cooled to about 50°C in the water bath, 2.5 mL of 4 M $MgCl_2$ were added. Serial 10-fold dilutions from -1 to -6 were made of the pre-treatment influent, post-chitosan treatment influent, and post filtration effluent in phosphate buffer for MS2 plaque assay by the DAL method.

The *E. coli* F_{amp} host for MS2 infectivity assay was prepared by inoculating a scrape (~200 μL) of frozen *E. coli* F_{amp} culture into 50 mL of TSB with 0.5 mL of Strep/Amp 100x antibiotic stock and incubating the suspension for 18-24 hours at 37°C. A log-phase *E. coli* suspension was made by adding 0.5 mL of the *E. coli* F_{amp} overnight culture and 0.5 mL of Strep/Amp 100x stock concentrate to 50 mL of TSB and incubating it on a shaker (at 100 rpm) for 2 hours at 37°C. After the allotted time had passed, the log-phase culture was removed from the incubator and the OD_{520} (optical density) was measured using the spectrometer (SmartSpec Plus, Bio-Rad). The log-phase *E. coli* F_{amp} culture OD target was between 0.2 and 0.8, and once this level was reached, the log-

phase culture was ready to use. A 5% volume (250 µL) of the log phase *E. coli* F_{amp} broth culture and a 1% volume (50 µL) of the Strep/Amp 100x antibiotic stock concentrate solution was added per 5 mL of molten agar medium in each 10 mL glass tube. A 100 µL volume of the sample dilution was added to the 5 mL volume of the molten agar solution, exposed to a sterilization flame, swirled to mix, and then poured onto the bottom agar layer culture plate. After the agar set, the plates were inverted and incubated at 37°C for 18-24 hours. After the allotted incubation time, the MS2 plaques that formed on the lawn of the *E. coli* host were observed, counted, and recorded.

3.11 Physical-Chemical Parameters

Both turbidity (in NTU) and pH were tested for pre-treatment influent, post-treatment/pre-filtration, and post-filtration effluent for various concentrations of chitosan for both challenge waters. Differences in Log_{10} reductions of test microbes were calculated. Turbidity was measured with a turbidimeter (Hach 2100AN Turbidimeter, Hach, Loveland CO) and pH was measured with a pH meter (pH Meter Model 215 Denver Instrument Company) and a combination electrode.

3.12 Physical Size Analysis of Floc Particles

The particle size analyzer, Mastersizer 3000 by Malvern, was used to analyze floc formation and size characterization resulting from coagulation-flocculation by the addition of 10 mg/L of chitosan acetate to the two challenge waters (natural lake water and natural lake water amended with 1% pasteurized sewage in lake water by volume). The Mastersizer has the ability to detect particle sizes ranging from 0.01 µm – 3,500 µm. One liter of the challenge water was placed in a beaker with a revolving paddle blade for mixing. Tubing was attached to and from the Mastersizer and placed in the water-filled beaker, secured with tape. One of the tubes is attached to a peristaltic pump that pushes the water in and out of the Mastersizer. The settings, as detailed in **Table 3.3**, were set prior to running the sample.

Table 3.3 Mastersizer 3000 by Malvern settings for chitosan acetate coagulation-flocculation and sedimentation experiments using three stirring conditions.

Parameter		Setting		
Identification	Material Name	Oleic Acid (approximate equivalent)		
	Particle Type	Non-Spherical		
	Refractive Index	1.433		
	Absorption Index	0.001		
	Density (g/cm^3)	1.002		
Dispersant	Dispersant Name	Water		
	Refractive Index	1.33		
Duration (seconds, s)	Stirring Procedure	Standard	Intermediate	Minimal
	Red Background measurement (s)	10		
	Red Sample measurement (s)	10		
	Blue Background measurement (s)	5		
	Blue Sample Measurement duration (s)	5		
	Number of measurements	64		
	Delay between measurements (s)	13	14	0
	Pre-measurement delay (s)	0	0	0
Obscuration	Obscuration Lower Limit (%)	2		
	Obscuration Upper Limit (%)	20		

After settings were established, the Mastersizer was initialized, background measurements were taken, and sample measurements were taken. The mixing procedures that were used for microbial assessments were also used in these experiments, as detailed in **Table 3.3**. Non-pasteurized-sewage amended raw challenge water was passed through the Mastersizer with the addition of 10 mg/L of chitosan acetate. The bottom 10^{th} (*Dx(10)*), 50^{th} (*Dx(50)*), and 90^{th} (*Dx(90)*) percentile floc size was measured and recorded over the entire coagulation-flocculation and sedimentation process. After sampling was complete, soapy water and deionized water was run through the tubing and the Mastersizer to remove any residual flocs or natural lake water. Deionized water was passed through the tubing, and tube endings were submerged in deionized water to prevent the Mastersizer measurement cell from drying out. All experiments were run in triplicate.

3.13 Statistical Analysis

A linear regression analysis was conducted among *E. coli* KO11, MS2 coliphage, and turbidity reductions by pooling data points by parameters—challenge water type, dose, and stirring condition, and sampling point. Estimate mean difference, standard error, t-values, p-values, and 95% confidence intervals of Log_{10} reductions were reported. The last 30 data points per PSA experiment were pooled by stirring condition and challenge water into sets of $n=90$; a t-test for parametric datasets was used to test for significant differences among challenge waters and stirring conditions. Statistical analyses were done using R-Studio.

CHAPTER 4: RESULTS

Table 4.1 Quality parameters and values for raw University Lake water samples used in experiments[1]

Parameter	Units	Average ± 95% CI	
		Sampled August 18, 2018	Sampled March 1, 2019
Turbidity	NTU	6.21±0.72	23.6±0.32
pH	-	7.57±0.29	6.89±0.02
Alkalinity[2]	Mg/L CaCO3	30	17.167±0.32
Total Organic Content	mg/L	8.81	5.94
Dissolved Oxygen Content	mg/L	7.67	5.19
UV254	cm-1	0.242	0.198
Total Coliform	MPN/100 mL	408	308
E. *coli*	MPN/100 mL	<4	50
Specific Conductance	µmohs/cm	87	65.5
Fluoride	mg/L	<0.10	<0.10
Manganese	mg/L	0.282	0.095
Iron	mg/L	0.91	0.53
Hardness	mg/L CaCO3	31±1.39	20

[1]Water for microbial experiments and for particle size analysis of test water with no added sewage was sampled on August 18, 2019. Water used for particle size analysis for sewage-amended test water was sampled on March 1, 2019. Water quality measurements were reported by OWASA.
[2] OWASA measures alkalinity as a raw water blend of 25% University Lake.

Table 4.2 LRVs and associated 95% confidence intervals for *E. coli* KO11 and MS2 coliphage per challenge water type, stirring condition, and various points of water sampling, in waters with and without chitosan acetate pre-treatment.

Challenge Water Type	Stirring Condition	Treatment Sections[4]	LRV ± 95% Confidence Interval	
			E. coli KO11	MS2 Coliphage
Non-past. Sewage amended	Filtration Alone		0.10 ± 0.03	0.1 ± 0.03
	Standard[3]	Post CH	1.2 ± 0.3	1.7 ± 0.3
		CH to EF	1.9 ± 0.3	1.4 ± 0.6
		Effluent	3.1 ± 0.3	3.2 ± 0.6
	Intermediate[3]	Post CH	1.2 ± 0.2	1.5 ± 0.2
		CH to EF	2.4 ± 0.8	1.9 ± 0.3
		Effluent	3.6 ± 0.8	3.4 ± 0.2
	Original[3]	Post CH	0.8 ± 0.2	1.8 ± 0.4
		CH to EF	2.3 ± 0.5	1.1 ± 0.4
		Effluent	3.2 ± 0.5	3.2 ± 0.4
1% past. Sewage amended	Filtration Alone		0.1 ± 0.1	0.4 ± 0.3
	Standard[3]	Post CH	1.3 ± 0.3	2.4 ± 0.3
		CH to EF	2.1 ± 0.3	1.3 ± 0.3
		Effluent	3.4 ± 0.3	3.7 ± 0.3
	Intermediate[3]	Post CH	1.4 ± 0.2	2.4 ± 0.2
		CH to EF	2.5 ± 0.5	1.1 ± 0.3
		Effluent	4.1 ± 0.5	3.6 ± 0.3
	Original[3]	Post CH	0.9 ± 0.1	1.3 ± 0.2
		CH to EF	2.3 ± 0.6	2.3 ± 0.3
		Effluent	3.2 ± 0.6	3.5 ± 0.3

[3]Dosed with 10 mg/L of chitosan acetate pre-treatment using the associated stirring condition
[4]Post CH = reductions due to chitosan pre-treatment alone; CH to EF = reductions due to filtration alone after pre-treatment; Effluent = reductions due to pre-treatment and filtration together

The average Log_{10} Reduction Values (LRVs) and 95% confidence intervals for *E. coli* KO11 in non- and 1% pasteurized sewage-amended challenge waters are displayed in **Figure 4.1** and **Figure 4.2**, respectively. Average LRVs and 95% confidence intervals for MS2 coliphage in non- and 1% pasteurized sewage-amended challenge waters are displayed in **Figure 4.3** and **Figure 4.4**, respectively. **Figure 4.1-4.4** group average LRVs based on challenge water type (non-pasteurized sewage amended samples and 1% pasteurized sewage amended samples), stirring condition (Standard, Intermediate, and Minimal as described in **Table 3.2**), and reductions due to

1) filtration, 2) chitosan pre-treatment, 3) filtration alone after pre-treatment, and 4) chitosan pre-treatment and filtration. Reductions due to filtration only after chitosan pre-treatment were calculated by subtracting chitosan pre-treatment reductions from overall reductions produced by chitosan pre-treatment and filtration together.

Table 4.6 summarizes statistical findings from a linear regression analysis comparing challenge water types, stirring conditions, and LRVs from various sampling points. All raw LRV data can be found in the **appendix D**.

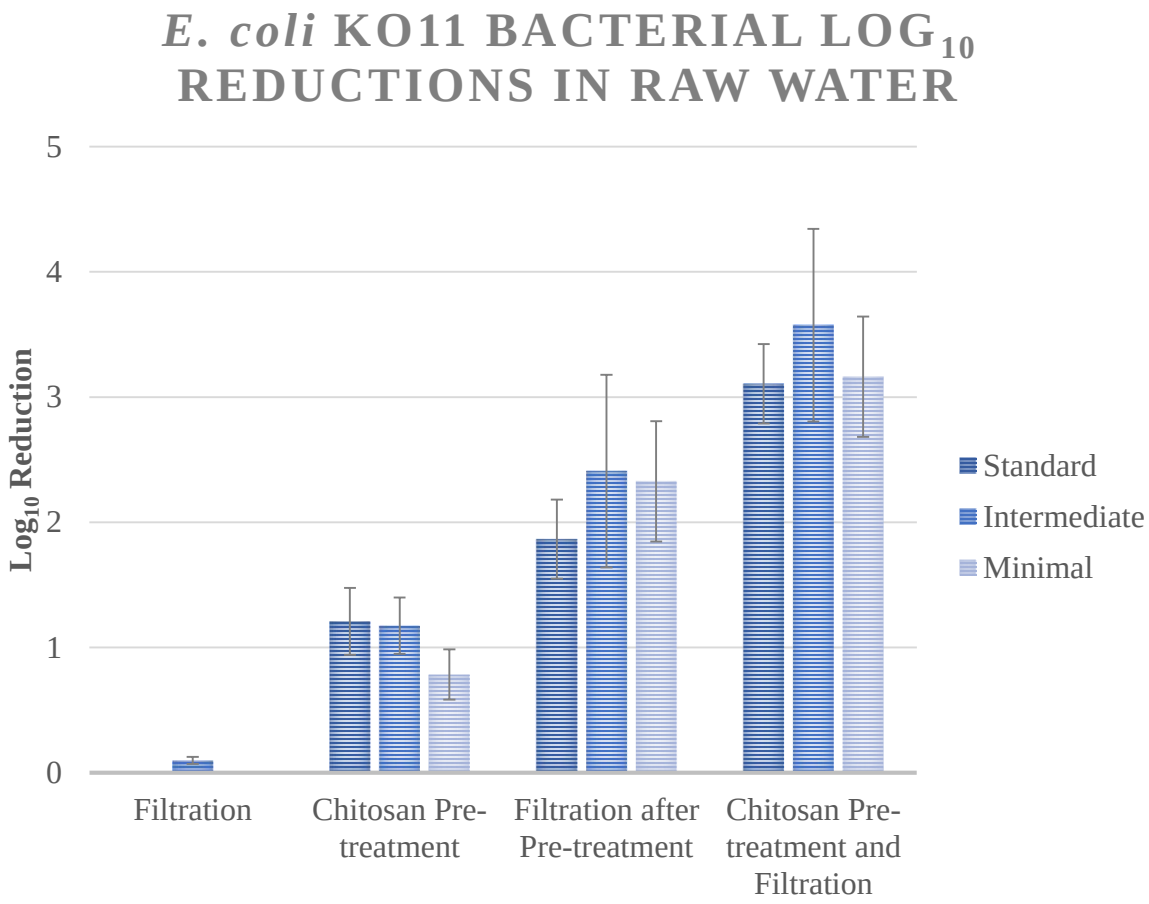

Figure 4.1 Averages and 95% confidence intervals (whisker lines) of E. *coli* KO11 LRVs in non-pasteurized sewage-amended samples from filtration alone and filtration after 10 mg/L chitosan acetate coagulation pre-treatment.

Filtration alone (without pre-treatment) resulted in an average *E. coli* KO11 LRV of 0.1 ± 0.03 in non-sewage amended water and 0.1 ± 0.1 in sewage-amended water. Chitosan acetate

coagulation-flocculation pre-treatment alone in non-pasteurized sewage-amended waters, for the three different stirring conditions resulted in varying LRVs. Minimal stirring conditions produced the lowest LRV (0.7 ±0.201) and intermediate and standard stirring conditions resulted in LRVs slightly above 1.0 (**Table 4.2**). All three stirring conditions, with non-sewage amended challenge waters, resulted in average bacterial LRVs greater than 3.0. Intermediate stirring conditions resulted in the highest LRV (3.6±0.8), and standard and minimal conditions averaged somewhat lower, with 3.1±0.3 and 3.2±0.5 LRVs, respectively.

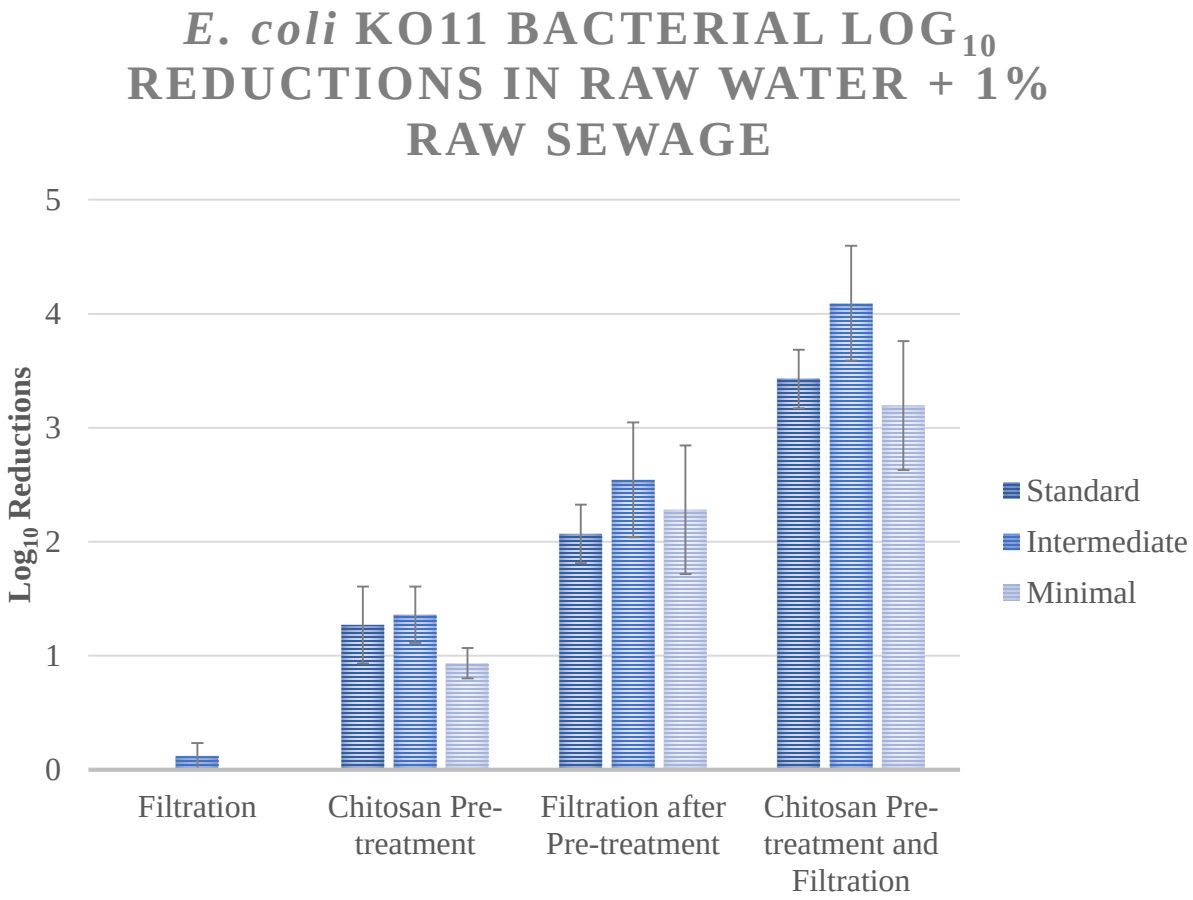

Figure 4.2 Average (bars) and 95% confidence intervals (whisker lines) of E. *coli* KO11 LRVs in 1% pasteurized sewage-amended water samples from filtration alone and filtration after coagulation-flocculation pre-treatment with 10 mg/L chitosan acetate.

E. coli KO11 LRVs in 1% pasteurized sewage-amended water samples appeared to follow a similar trend as found in non-sewage amended water samples. The minimal stirring

conditions had the lowest average LRV due to chitosan coagulation-flocculation pre-treatment alone (0.9±0.1). Chitosan pre-treatment alone for the standard and intermediate stirring conditions resulted in average LRVs of 1.3±0.3 and 1.4±0.2, respectively. Average bacterial reductions due to filtration alone, after pre-treatment, ranged from 2.1 – 2.5 Log_{10}, with intermediate stirring producing the highest average LRV. Bacterial reduction as a result of pre-treatment, intermediate stirring conditions, and filtration in 1% pasteurized sewage-amended raw waters were observed to be 4.1±0.5. The corresponding experiments associated with standard and minimal stirring conditions resulted in somewhat lower reductions, yet still achieved greater than 3 Log_{10} reductions.

When controlling for all other parameters, average *E. coli* KO11 LRVs in 1% pasteurized-sewage amended raw water samples were found to be 0.14 higher than LRVs in non-pasteurized sewage-amended raw water samples. Although a small value, this difference in LRVs was found to be significant at a 90% confidence level ($p = 0.094$).

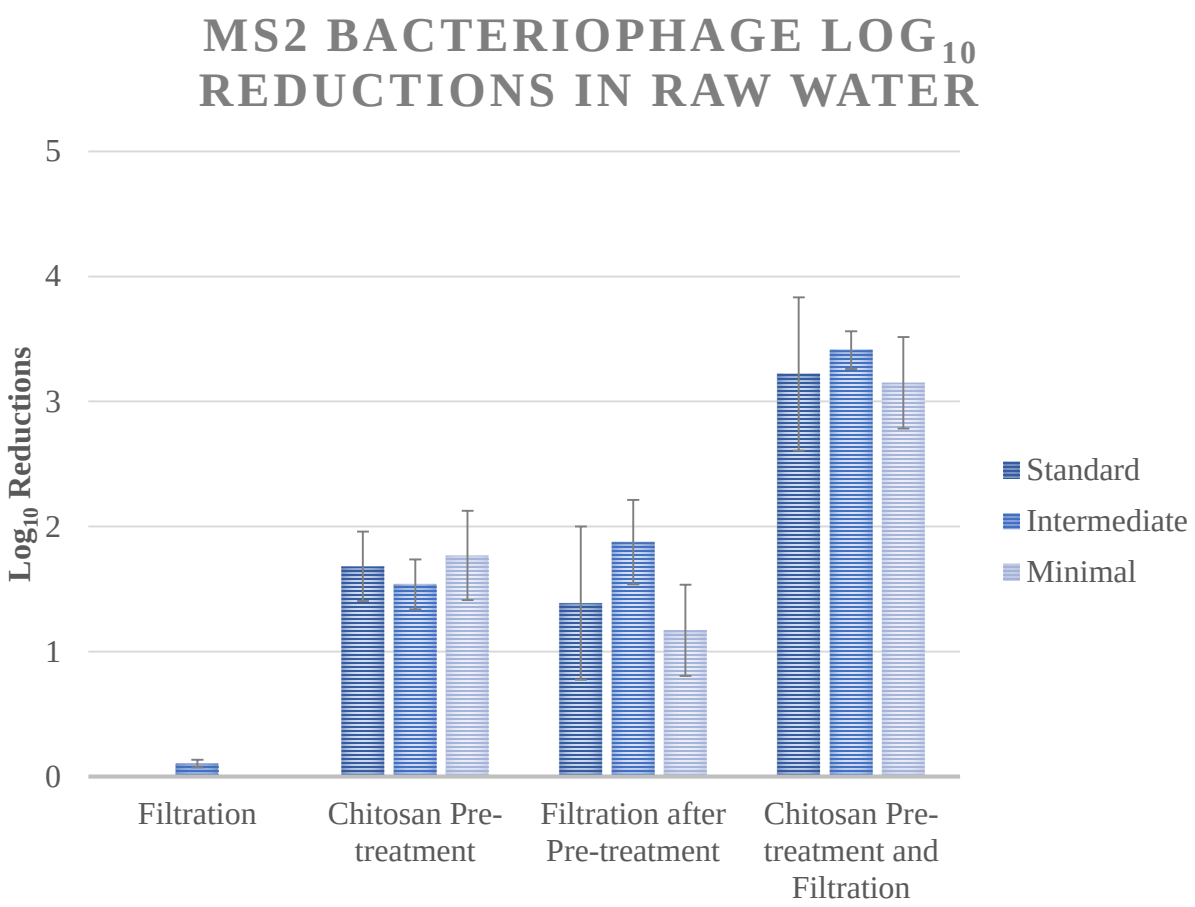

Figure 4.3 Average (bars) and 95% confidence intervals (whisker lines) of MS2 coliphage LRVs in non-pasteurized sewage-amended samples from filtration alone and filtration after coagulation-flocculation pretreatment with10 mg/L chitosan acetate.

MS2 coliphage LRVs due to filtration (after chitosan pre-treatment) were on average 0.30 LRVs higher than reductions due to chitosan pre-treatment alone (*p-value < 0.022)*, and 1.31 LRVs higher than reductions due to filtration alone (*p-value < 0.00001)*. Reductions due to the combined effects of chitosan pre-treatment and filtration were, on average, 3.21 Log_{10} reductions higher than filtration alone (*p-value < 0.00001)* (**Table 4.6**).

Average MS2 coliphage reductions followed a similar trend as bacterial reductions in both non- and 1% sewage-amended raw water samples. Filtration alone (no pre-treatment) resulted in average LRVs of 0.1±0.03 and 0.4±0.3 for non- and 1% sewage-amended challenge waters, respectively (**Table 4.2**). In non-sewage amended challenge waters, pre-treatment alone

produced similar average LRVs among all three stirring conditions, ranging from 1.5 – 1.8 Log_{10} reductions. However, average MS2 coliphage reductions due to chitosan coagulation-flocculation pre-treatment in 1% pasteurized sewage amended samples varied, with standard and intermediate stirring samples resulting in average LRVs greater than 2 but minimal stirring samples achieving only about 1 LRV. All average MS2 coliphage Log_{10} reductions due to pre-treatment and filtration, regardless of stirring condition or challenge water type, resulted in average LRVs greater than 3. Average LRVs for standard, intermediate, and minimal stirring conditions in 1% pasteurized sewage-amended samples were all higher than corresponding average LRVs of non-pasteurized sewage -mended samples, as seen in **Figure 4.3** and **Figure 4.4.**

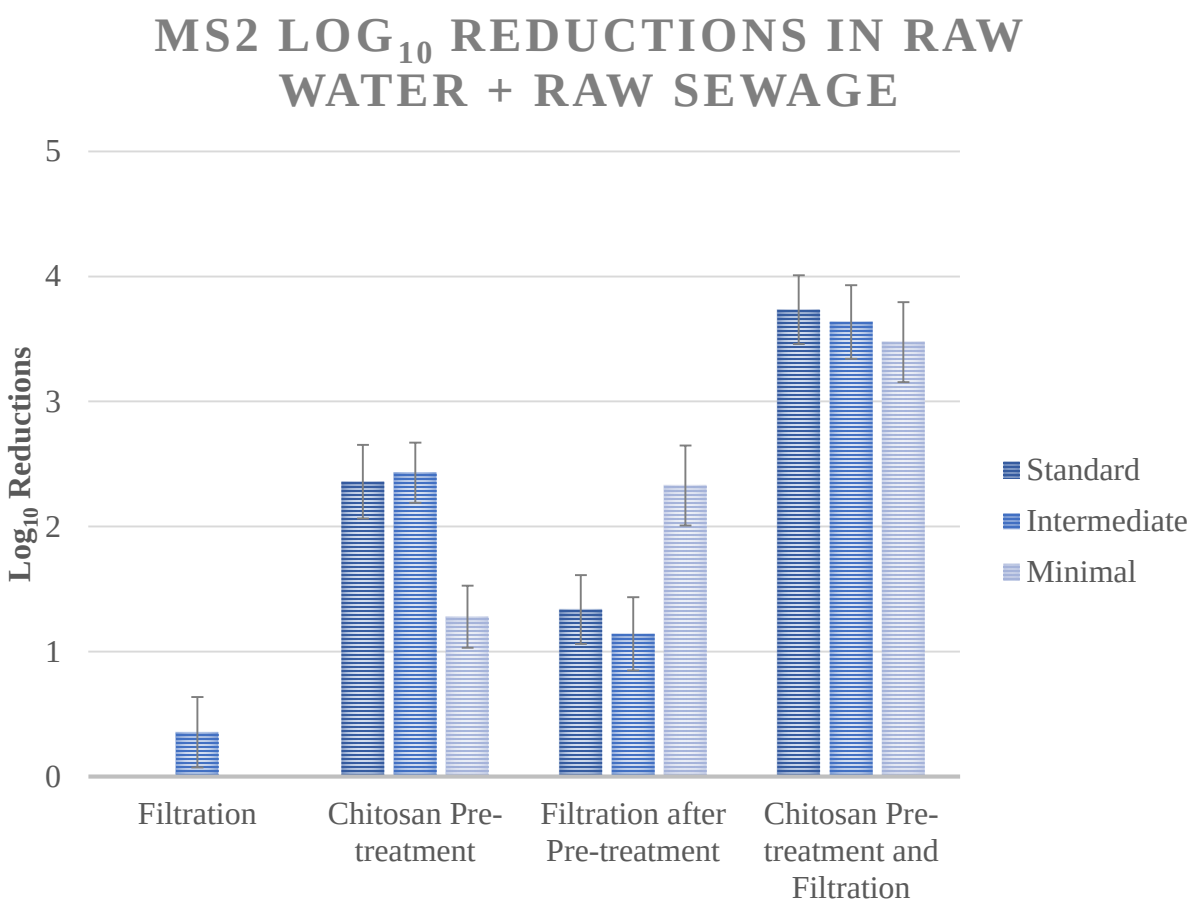

Figure 4.4 Average (bars) and 95% confidence intervals (whisker lines) of MS2 coliphage LRVs in 1% pasteurized sewage-amended samples from filtration alone and filtration after chitosan-flocculation pretreatment with 10 mg/L chitosan acetate.

When controlling for all other parameters, average MS2 coliphage LRVs of 1% pasteurized sewage-amended samples were found to be 0.27 Log_{10} reductions higher than non-pasteurized-sewage amended samples, which was significant at a 99% confidence level (*p = 0.0040)*. Additionally, MS2 coliphage LRVs due to filtration alone (but after pre-treatment), were on average, 0.3 Log_{10} reductions lower than reductions due to chitosan pre-treatment alone (*p-value < 0.022)*, and 1.3 Log_{10} reductions higher than reductions due to filtration alone (*p-value < 0.00001)*. Reductions due to the combined efforts of chitosan pre-treatment and filtration were, on average, 3.3 Log_{10} reductions higher than filtration alone (*p-value < 0.00001)* (**Table 4.6**).

Table 4.3 Raw Turbidity values for each sampling point by stirring condition and test water.

		Raw Turbidity Values (NTU)				
Test Water	Stirring Conditions	Raw Lake Water	Influent	Filtration Alone	Pre-treated	Effluent
No Sewage	Standard	3.6	3.6	2.5	0.90	0.31
						0.25
						0.30
	Intermediate	8.6	11.3	2.5	3.7	0.53
						0.47
						0.48
	Original	7.1	7.4	6.2	6.4	0.81
						0.90
						0.79
Sewage	Standard	5.0	7.2	2.3	1.4	0.80
						0.35
						0.40
	Intermediate	5.9	7.6	3.3	3.2	0.71
						0.30
						0.55
	Original	7.1	8.7	7.6	6.4	0.66
						0.85
						0.52

Table 4.4 Raw pH values for each sampling point by stirring condition and test water.

		Raw pH Values				
Test Water	Stirring Conditions	Raw Lake Water	Influent	Filtration Alone	Pre-treated	Effluent
No Sewage	Standard	7.1	7.0	7.4	7.0	7.0
						7.4
						7.7
	Intermediate	8.9	8.7	9.0	8.8	8.5
						8.5
						8.6
	Original	7.2	7.2	7.5	7.2	7.3
						7.5
						7.3
Sewage	Standard	7.5	7.5	7.6	7.5	7.6
						7.8
						8.4
	Intermediate	7.4	7.4	7.7	7.5	7.8
						7.7
						8.1
	Original	7.2	7.2	7.3	7.3	7.6
						7.3
						7.4

Table 4.5 Average percent changes (and 95% confidence limits) in pH and Log_{10} reductions in turbidity for the two types of challenge waters, three different stirring conditions, and filtration alone.

Challenge Water	Stirring Condition	Average ± 95% Confidence Interval*	
		pH (%)	Turbidity LRV
Non-pasteurized-sewage water	Filtration Alone	4.2±0.93	0.3±0.36
	Standard	5.0±5.3	1.1±0.1
	Intermediate	-1.1±1.1	1.4±0.03
	Minimal	2.0±1.6	1.0±0.03
1% pasteurized-sewage water	Filtration Alone	1.8±1.5	0.3±0.3
	Standard	5.6±6.	1.2±0.2
	Intermediate	6.3±3.5	1.2±0.2
	Minimal	3.3±1.9	1.1±0.1

***** Changes and reductions are due to pre-treatment and filtration together.

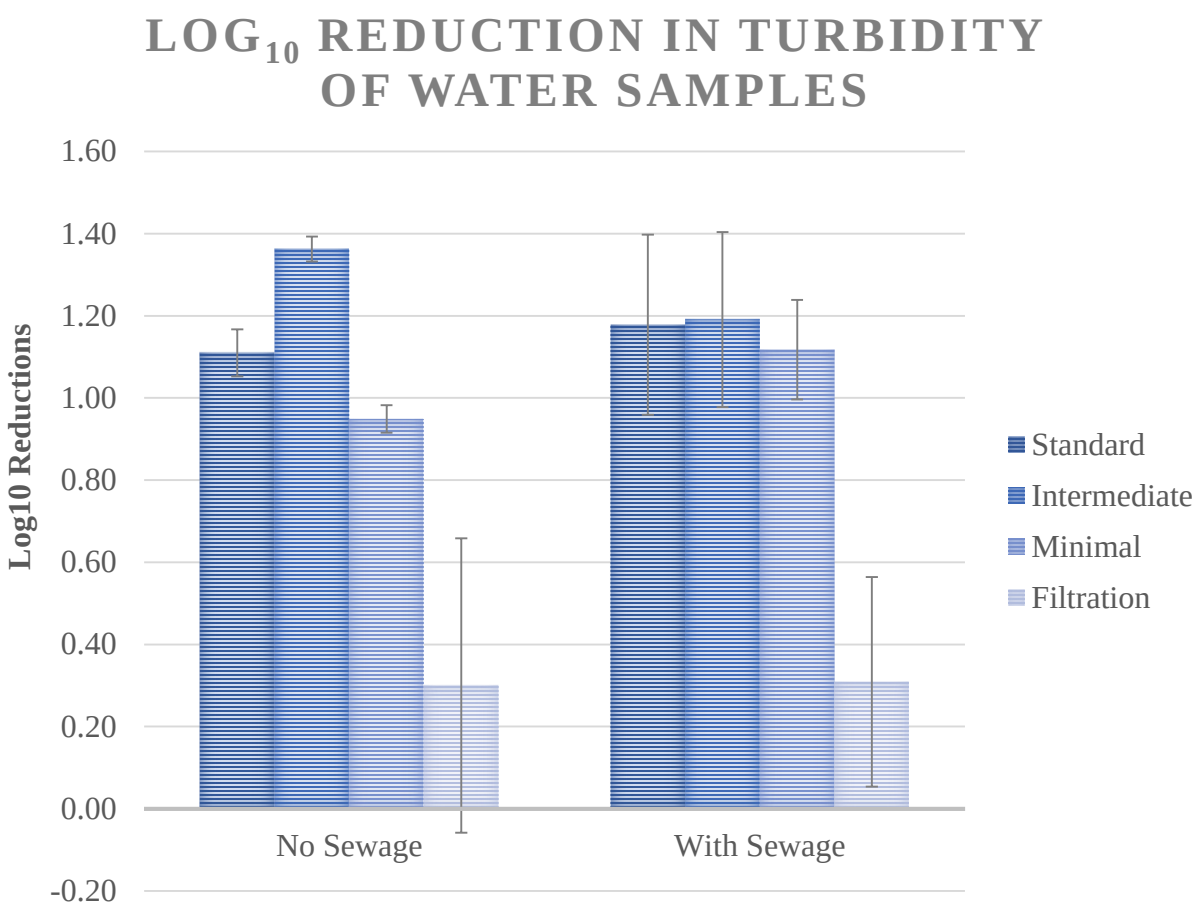

Figure 4.5 Average and 95% confidence intervals of Turbidity LRVs in raw water samples and with and without 1%-sewage among three different stirring conditions, standard, intermediate, minimal, and filtration alone.

Average Log_{10} reductions and associated 95% confidence intervals in turbidity are summarized in **Figure 4.5**. Average reductions were grouped by challenge water type as well as the absence and presence of chitosan coagulation-flocculation pre-treatment and different flocculation stirring conditions. Bars labeled "Standard," "Intermediate," and "Minimal" represent reductions in turbidity due to filtration of pre-treated water with the use of three different stirring conditions as described in **Table 3.2**. Bars labeled "Filtration" were not subject to pre-treatment and had the lowest average LRV for both challenge water types: 0.3 ± 0.36 for non-pasteurized sewage amended raw water samples and 0.3±0.3 for 1% pasteurized sewage amended raw water samples. Turbidity Log_{10} reductions ranged from 0.96 – 1.36 for non-

pasteurized-sewage samples and 1.12 – 1.19 for 1% pasteurized sewage amended water samples. Intermediate stirring conditions resulted in the highest turbidity reduction for both challenge water types. All experiment triplicates, for all three stirring conditions of chitosan coagulation-flocculation pre-treatment with 10 mg/L of chitosan acetate in both challenge waters resulted in raw effluent turbidity values that were consistently > 1 NTU. All non-pretreated experiment triplicates, where only filtration was employed, gave raw effluent turbidity values consistently above 1 NTU (**Table 4.3**).

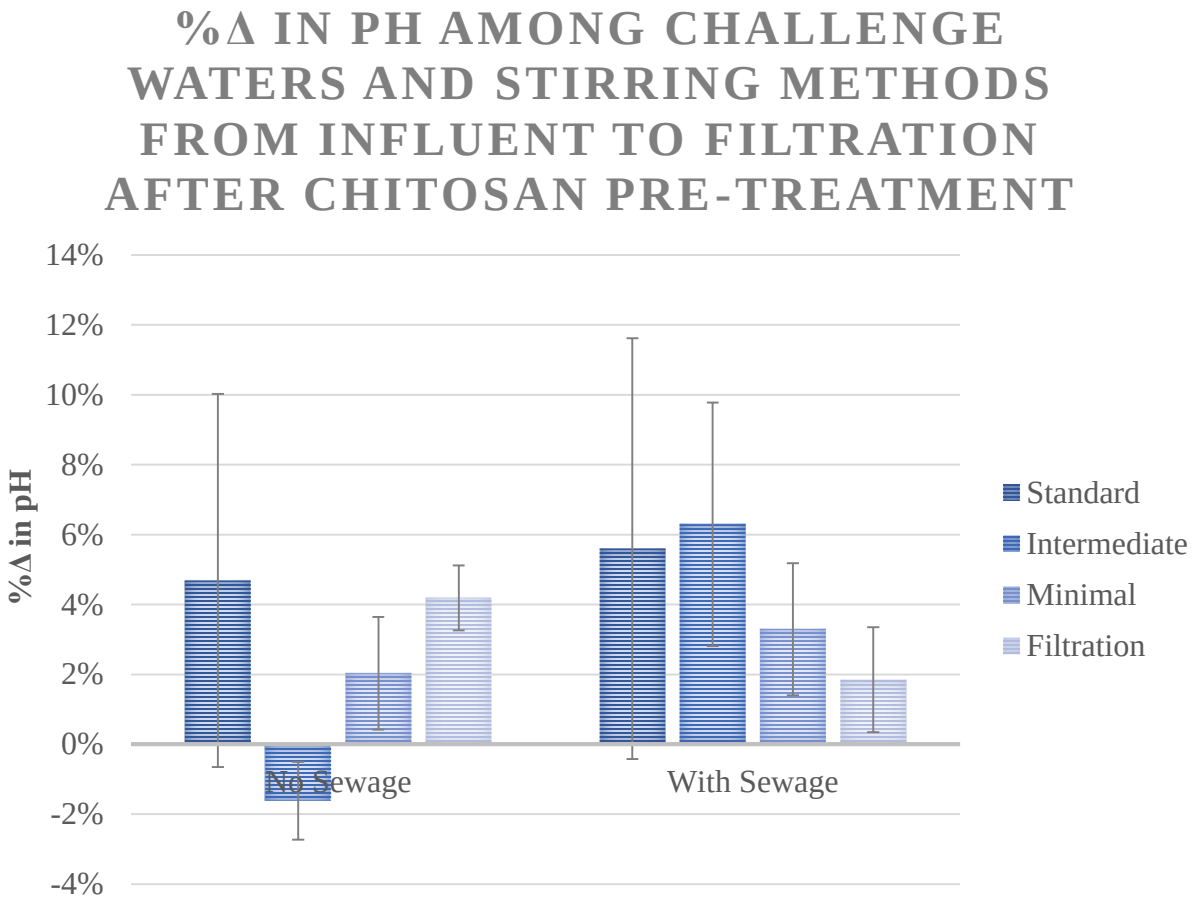

Figure 4.6 Average percent change and 95% confidence limits in pH among1% and non-pasteurized sewage amendment water samples, and the three coagulation-flocculation stirring conditions (standard, intermediate, and minimal) and filtration alone.

Average turbidity LRVs from intermediate stirring conditions and filtration had a mean difference of 0.24 Log_{10} reductions above minimal stirring conditions and filtration (*p-value* =

0.025). Average Log_{10} turbidity reductions due to pre-treatment with intermediate, standard, and minimal coagulation-flocculation stirring conditions and filtration were all found to be significantly higher than Log_{10} turbidity reductions achieved by filtration alone, achieving 0.98 LRV (*p-value < 0.00001*), 0.84 LRV (*p-value < 0.00001*), and 0.73 LRV (*p-value < 0.00001*), respectively **Table 4.6**).

Table 4.6 Comparisons and estimated mean differences in average LRVs of *E. coli* KO11, MS2 coliphage, and turbidity for comparison parameters listed.[5]

	Comparison		Estimated LRV Mean Difference	Standard Error	t-value	Pr(>|t|)
Post Chitosan	MS2	*E. coli* KO11	0.72	0.117	9.738	< 0.000010***
***E. coli* KO11**	Sewage	No Sewage	0.14	0.080	1.70	0.094’
	Intermediate	Minimal	0.31	0.10	3.15	0.0024**
	Intermediate	Standard	0.28	0.10	2.82	0.0064**
	Standard	Minimal	0.03	0.10	0.34	0.74
	CHEF	Pre-treatment	1.13	0.11	9.97	< 0.000010***
	INEF	Pre-treatment	2.30	0.11	20.39	< 0.000010***
	Pre-treatment	Filtration	1.01	0.11	8.96	< 0.000010***
	INEF	CHEF	1.18	0.11	10.42	< 0.000010***
	CHEF	Filtration	2.14	0.11	18.93	< 0.000010***
	INEF	Filtration	3.32	0.11	29.35	< 0.000010***
MS2 coliphage	Sewage	No Sewage	0.27	0.090	2.99	0.0040**
	Intermediate	Minimal	0.11	0.11	0.96	0.34
	Intermediate	Standard	0.04	0.11	0.35	0.73
	Standard	Minimal	0.07	0.11	0.62	0.54
	Pre-treatment	CHEF	0.30	0.13	2.35	0.022*
	INEF	Pre-treatment	1.59	0.13	12.36	< 0.000010***
	Pre-treatment	Filtration	1.61	0.13	12.50	< 0.000010***
	INEF	CHEF	1.90	0.13	14.71	< 0.000010***
	CHEF	Filtration	1.31	0.13	10.16	< 0.000010***
	INEF	Filtration	3.21	0.13	24.86	< 0.000010***
Turbidity	Sewage	No Sewage	0.02	0.070	0.26	0.80
	Intermediate	Minimal	0.24	0.10	2.43	0.025*
	Intermediate	Standard	0.13	0.10	1.31	0.20
	Intermediate	Filtration	0.98	0.10	9.73	< 0.000010***
	Standard	Minimal	0.11	0.10	1.11	0.28
	Minimal	Filtration	0.73	0.10	7.30	< 0.000010***
	Standard	Filtration	0.84	0.10	8.41	< 0.000010***

[5]LRVs were initially categorized based on parameters listed in the first column (Post Chitosan, *E. coli* KO11, MS2 coliphage, and Turbidity), and then further categorized to compare parameters listed in the second and third column. Parameters listed in the second column were

found to have higher LRVs than parameters listed in the third column. The estimated LRV mean difference between the two compared parameters are listed along with associated standard errors, t-values, and p-values (Pr(>|t|). *Standard*, *Intermediate*, and *Minimal* refer to the three stirring conditions. *Sewage* and *No Sewage* refer to pasteurized sewage-amended and non-amended challenge waters, respectively. *Filtration* refers to filtration alone without pre-treatment, *Pre-treatment* refers to pre-treatment alone, *CHEF* refers to the isolated effects of filtration after pre-treatment, and *INEF* refers to the effects of pre-treatment and filtration together.
‘α = 0.10; 90% confidence-level
* α = 0.050; 95% confidence-level
** α = 0.010; 99% confidence-level
*** α < 0.000010; > 99.999% confidence-level

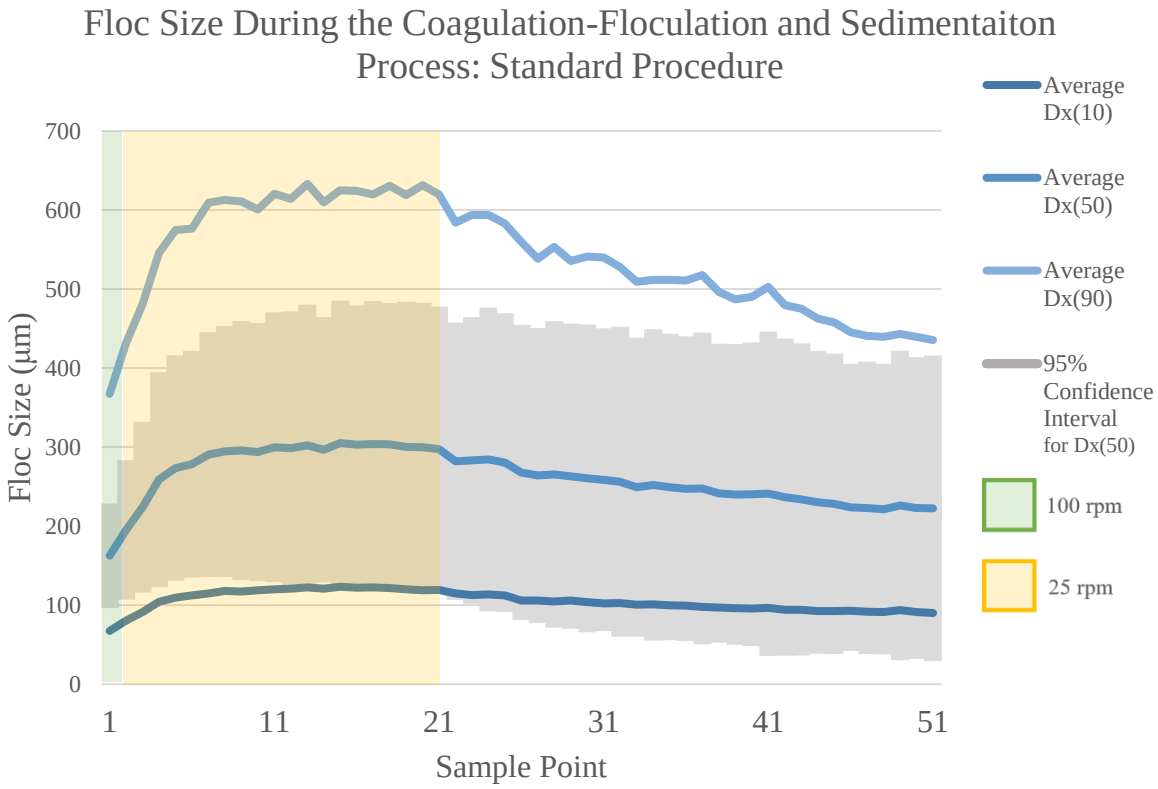

Figure 4.7 Floc size distribution over time with standard flocculation stirring procedures in non-sewage amended raw challenge waters—collected on August 18, 2018 (n = 51)

Results of triplicate experiments were averaged and the 95% confidence interval for Dx(50) was plotted. Green and yellow regions indicate measurements taken during various stirring speeds. The uncolored region indicates the final settling period of 30 minutes at 0 rpm. The median floc size (*Dx(50)*) for the standard stirring condition in challenge waters collected in August, 2018

ranged from 200 µm – 300 µm. However, the smallest 10% of flocs (*Dx(10)*) were approximately ≤100 µm (**Figure 4.7**).

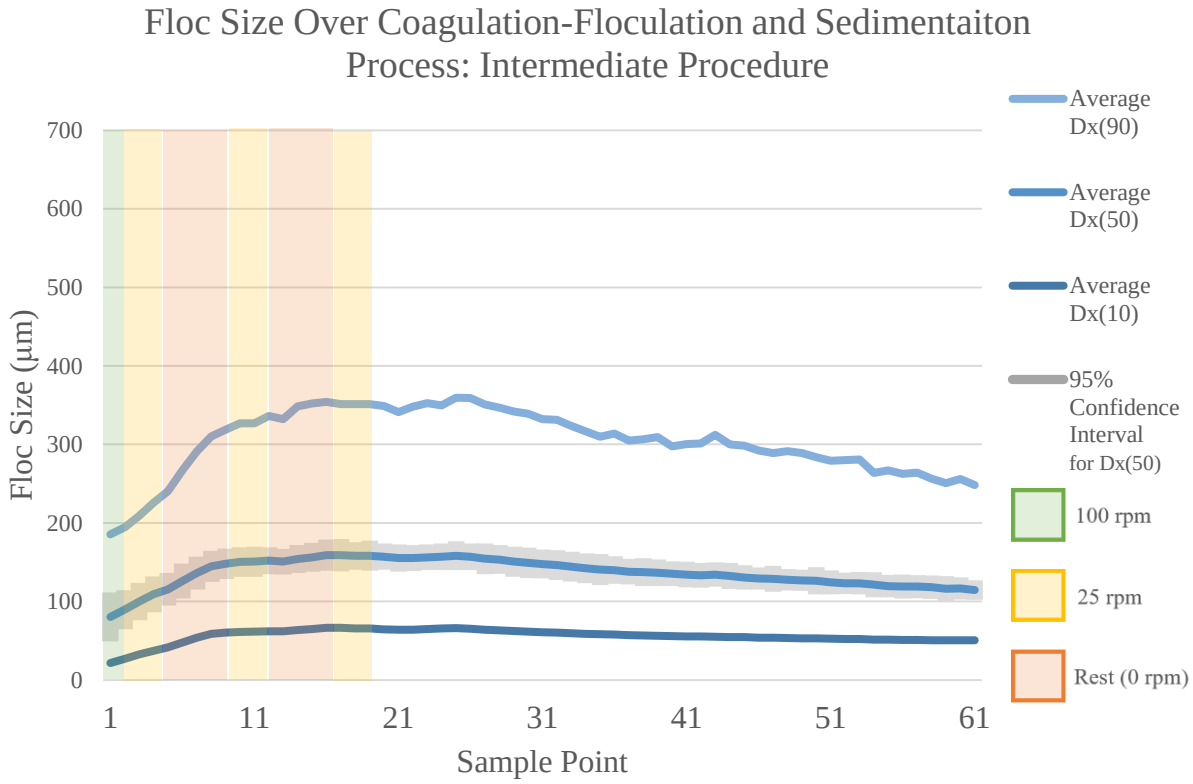

Figure 4.8 Floc size distribution over time with intermediate flocculation stirring procedures in non-sewage amended raw challenge waters—collected on August 18, 2018 (n=61).

Results of triplicate experiments were averaged and the 95% confidence interval for Dx(50) was plotted. Green, yellow, and red regions indicate measurements taken during various stirring speeds. The uncolored region indicates the final settling period of 30 minutes. The median floc size for the intermediate stirring condition in challenge waters collected in August, 2018 ranged from 100 µm – 175 µm. However, the smallest 10% of flocs (*Dx(10)*) were approximately ≤75 µm (**Figure 4.8**).

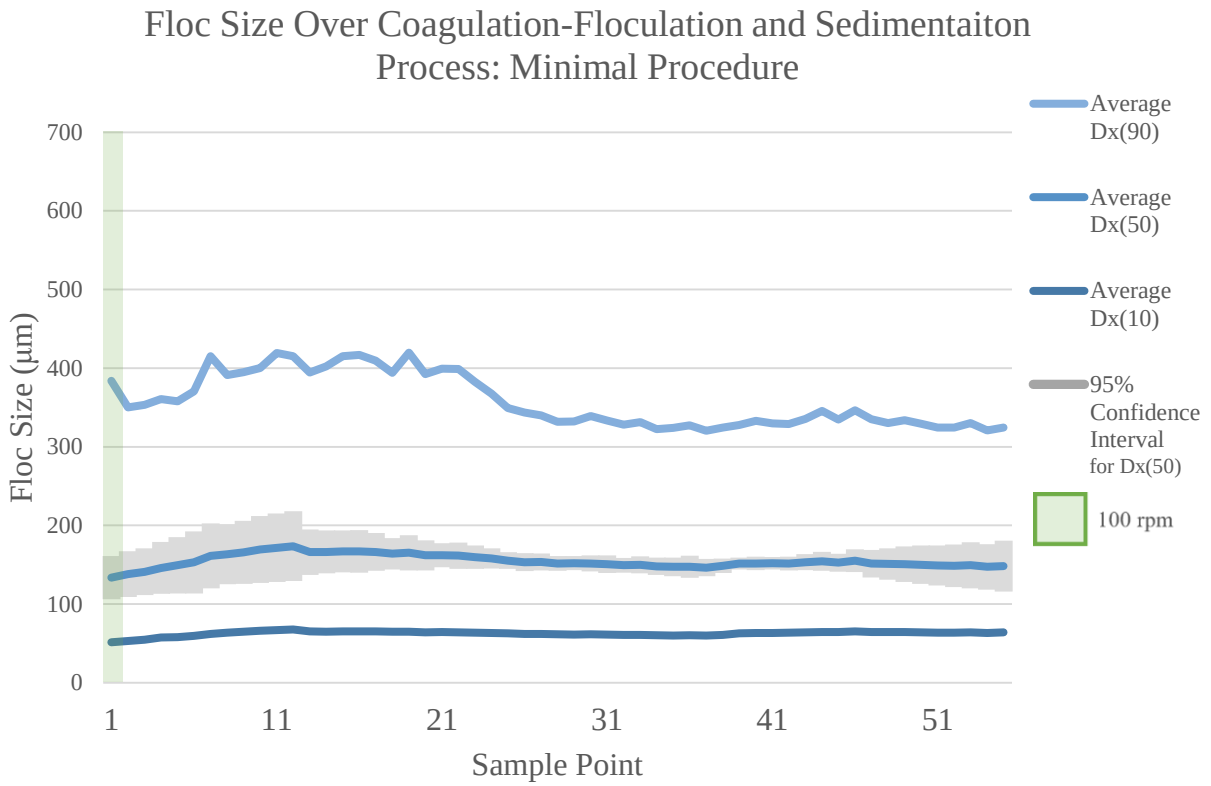

Figure 4.9 Floc size distribution over time with minimal flocculation stirring procedures in non-sewage amended raw challenge waters—collected on August 18, 2018 (n=55).

Results of triplicate experiments were averaged and the 95% confidence interval for Dx(50) was plotted. The green region indicates measurements taken during the 1-minute, 100 rpm stirring speed. The uncolored region indicates the end settling period of 30 minutes. The median floc size for the minimal stirring condition in challenge waters collected in August, 2018 ranged from 120 µm – 180 µm. However, the smallest 10% of flocs were approximately ≤75 µm (**Figure 4.9**).

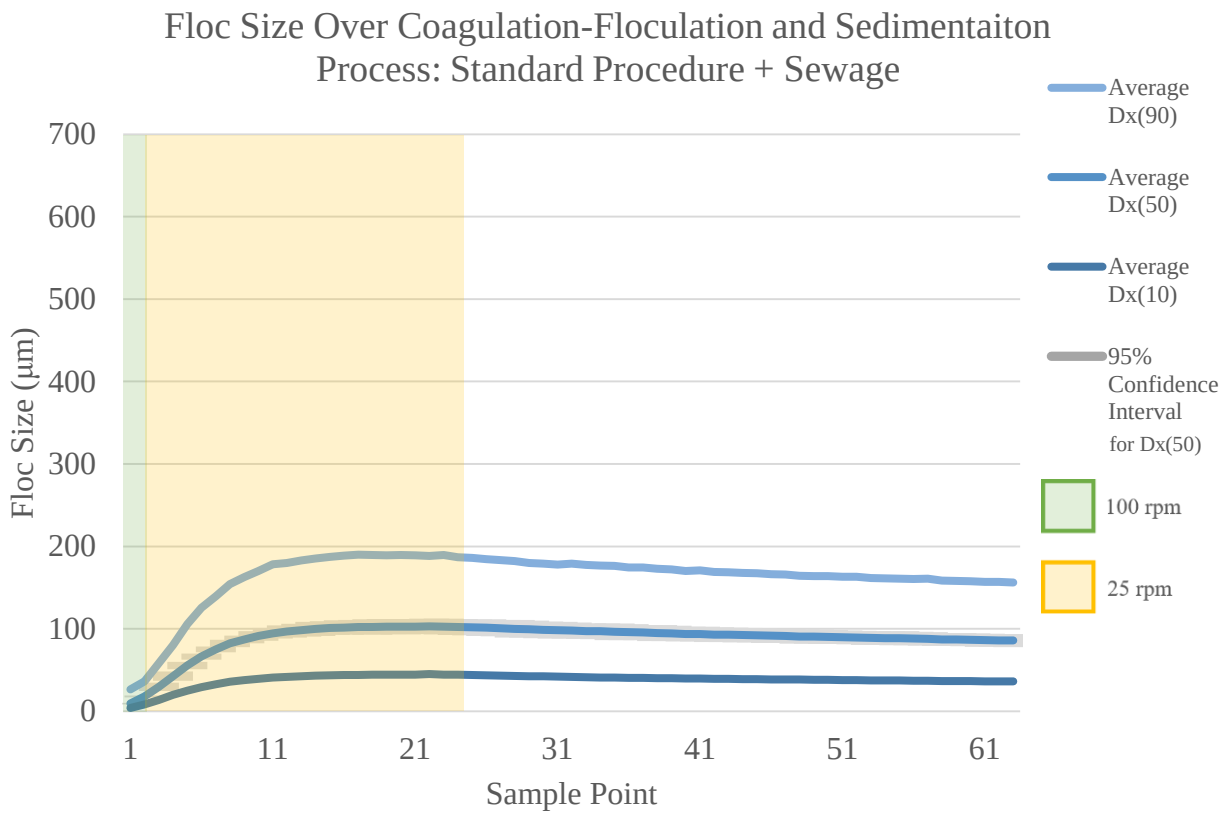

Figure 4.10 Floc size over coagulation-flocculation and sedimentation process with standard stirring conditions in 1% pasteurized sewage-amended challenge waters—collected on March 1, 2019 (n=63)

Results of triplicate experiments were averaged and the 95% confidence interval for Dx(50) was plotted. Green and yellow regions indicate measurements taken during various stirring speeds. The uncolored region indicates the end settling period of 30 minutes. The median floc size for the standard stirring condition in sewage amended challenge waters collected in March, 2019 was ~90 µm. However, the smallest 10% of flocs were approximately ≤50 µm (**Figure 4.10**).

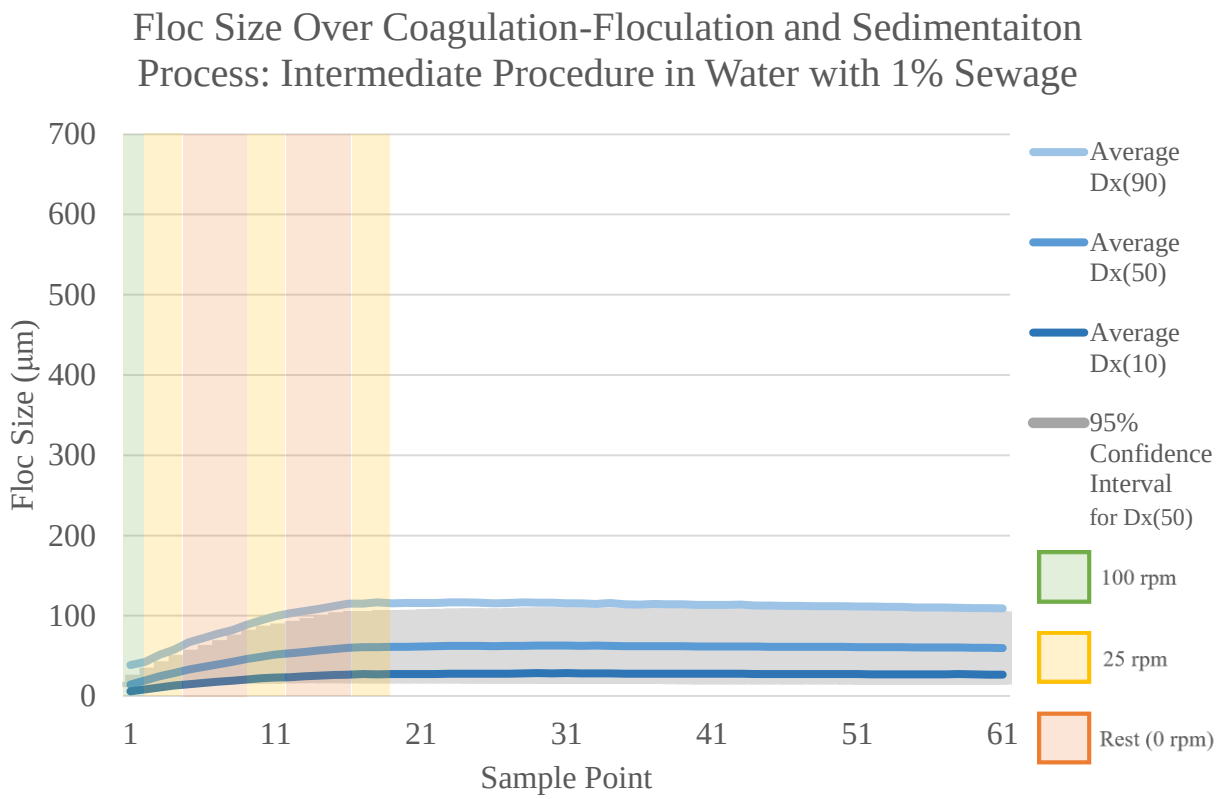

Figure 4.11 Floc size over coagulation-flocculation and sedimentation process with intermediate stirring conditions in 1% pasteurized sewage-amended raw challenge waters—collected on March 1, 2019 (n=61)

Results of triplicate experiments were averaged and the 95% confidence interval for Dx(50) was plotted. Green, yellow, red regions indicate measurements taken during various stirring speeds. The uncolored region indicates the end settling period of 30 minutes. The median floc size for the intermediate stirring condition in sewage amended challenge waters collected in March, 2019 was ~60 µm. However, the smallest 10% of flocs were approximately ≤30 µm (**Figure 4.11**).

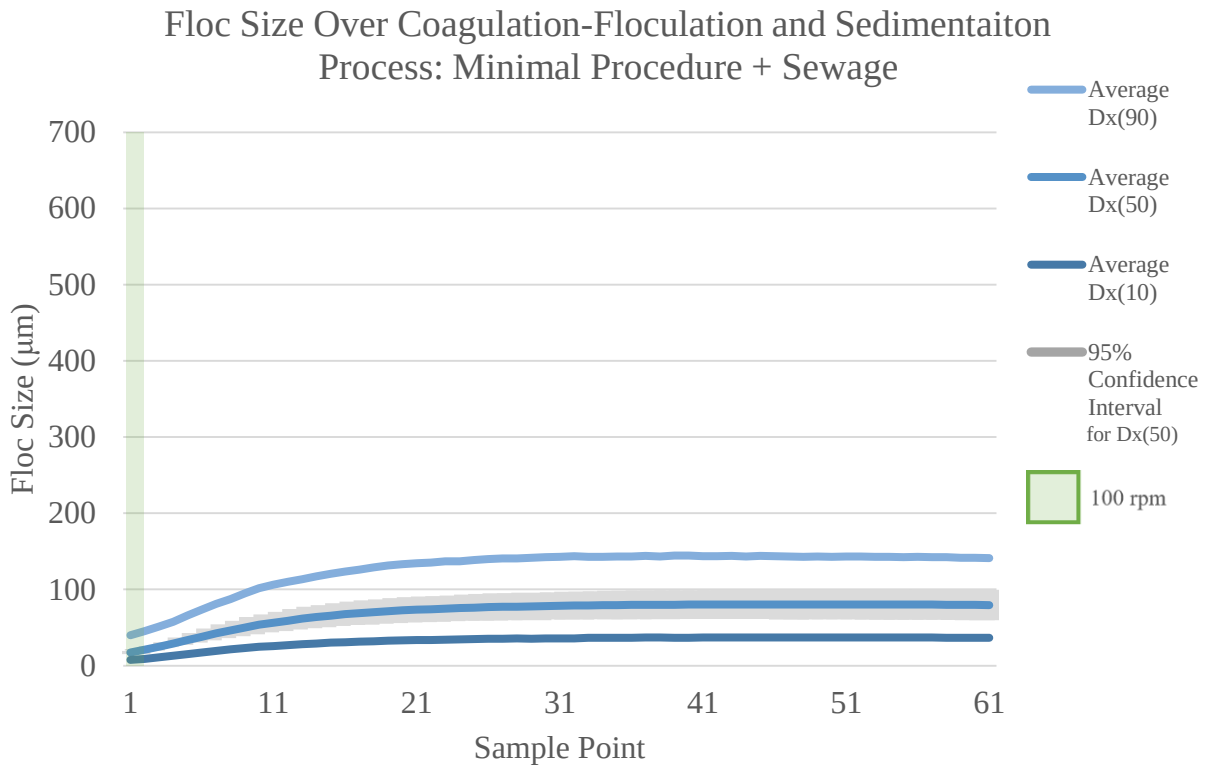

Figure 4.12 Floc size over coagulation-flocculation and sedimentation process with minimal stirring conditions in 1% pasteurized-sewage amended samples—collected on March 1, 2019 (n = 61)

Results of triplicate experiments were averaged and the 95% confidence interval for Dx(50) was plotted. The green region indicates measurements taken during the 1-minute, 100 rpm stirring speed. The uncolored region indicates the end settling period of 30 minutes. The median floc size for the minimal stirring condition in sewage amended challenge waters collected in March, 2019 was ~80 µm. However, the smallest 10% of flocs were approximately ≤40 µm (**Figure 4.12**).

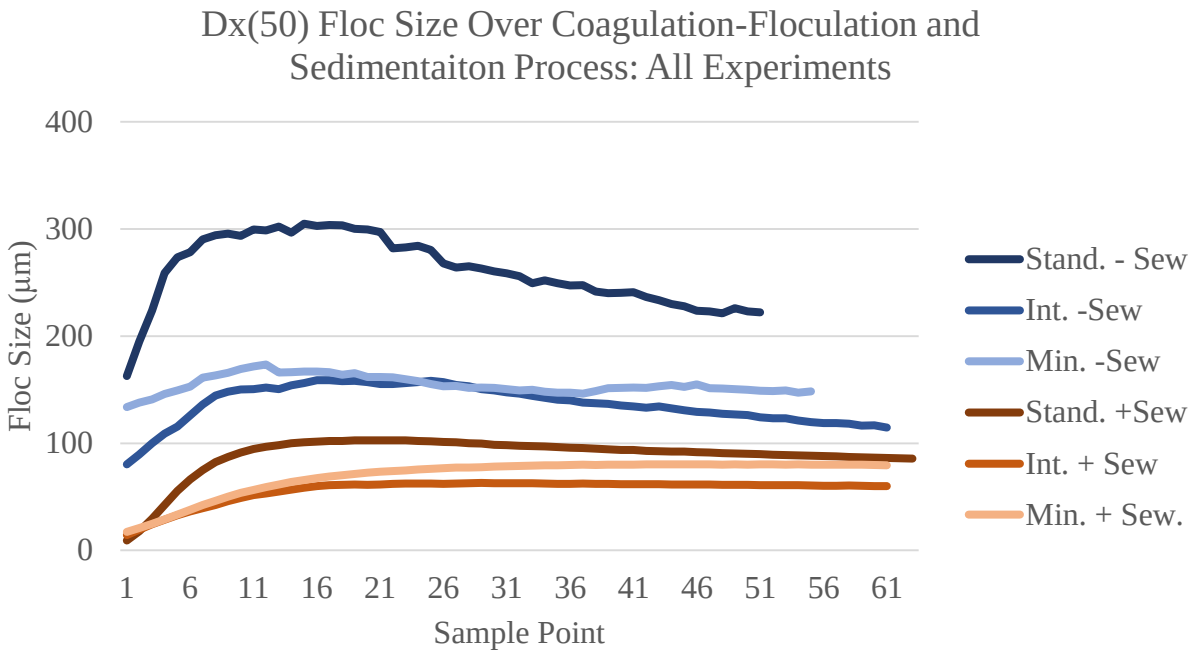

Figure 4.13 Average median (Dx(50)) floc measurements by stirring conditions and challenge water type over coagulation-flocculation sedimentation process.

Table 4.7 Average floc size and 95% confidence limits for the last 30 data points from each triplicate experiment per challenge water type and stirring condition.

Challenge Water Type	Stirring Condition	Average Floc Size ± 95% Confidence Interval
Non-Sewage-amended Water (Sampled August)	Standard	248±27
	Intermediate	129±3.0
	Minimal	150±3.2
1% Pasteurized-sewage-amended Water (Sampled March)	Standard	91±1.5
	Intermediate	61±7.0
	Minimal	80±2.9

The standard stirring conditions resulted the largest average floc size for both challenge waters, the intermediate stirring conditions produced the smallest average floc size for both challenge waters and the minimal mixing gave results floc between them (**Table 4.7**).

Table 4.8 T-tests for statistical significance between pairs of test water with and without added sewage and different mixing conditions during coagulation-flocculation and settling.[b]

Comparison		95% Confidence Interval of Mean Difference in Floc Particle Size	t-value	p-value
Non-sewage amended (August) > Sewage amended (March)	Standard	129.46, 184.87	11.27	< 0.000010*
	Intermediate	60.28, 75.71	17.45	
	Minimal	65.61, 74.21	32.11	
Non-sewage amended (August)	Standard > Intermediate	90.84, 146.49	8.47	
	Standard > Minimal	70.34, 126.02	7.00	
	Minimal > Intermediate	16.09, 24.89	9.19	
Sewage amended (March)	Standard > Intermediate	22.24, 36.75	8.07	
	Standard > Minimal	7.66, 14.17	6.63	
	Minimal > Intermediate	79.96, 61.38	4.80	

[b] The last 30 measurement points from each triplicate particle size analysis experiment were combined for a total sample size of 90 points per stirring condition and water sample type. Non-sewage PSA experiments were conducted with water sampled on August 18th, 2019, and sewage-amended PSA experiments were conducted with water sampled on March 1st, 2019. Combined sample points for non-sewage amended samples and sewage amended samples of the same stirring conditions were compared, as well as comparisons of different stirring conditions for the same challenge water samples, using a t-test for parametric analysis. The 95% confidence interval of mean difference among the two compared data sets, the t-value, and the associated p-values.
* $\alpha = 0.00001$; 99.999% confidence-level

The particle size analysis of chitosan flocs among challenge water types and stirring conditions were statistically compared in **Table 4.8**. The non-sewage amended water samples from the August collection had significantly higher floc sizes than the floc sizes from sewage-amended water samples from the March collection ($p < 0.000010$). Standard stirring procedures were found to produce flocs significantly larger than those produced from intermediate and minimal procedures for both challenge water types ($p < 0.000010$). Intermediate stirring conditions

produced significantly smaller flocs than standard and minimal stirring conditions for both challenge waters ($p < 0.000010$).

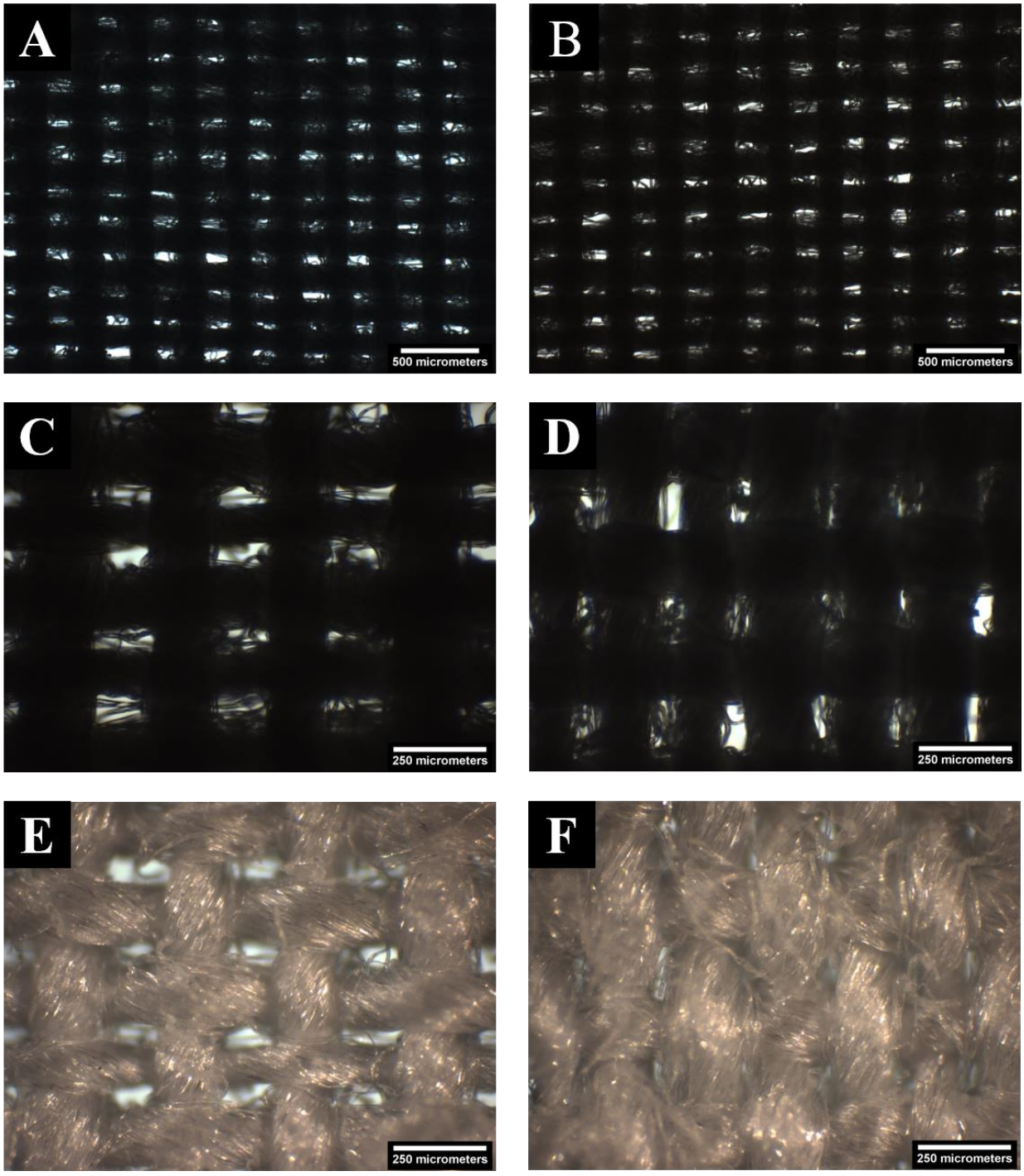

Figure 4.14 100% cotton cloth filter material under a light microscope

New (boxes A, C, E) and used (boxes B, D, F) cloth filter material was observed under a light microscope to measure the approximate pore size (~100 μm). The fibers of the used cloth are visibly agitated and frayed (box F), as compared to the new material (box E) (**Figure 4.14**).

CHAPTER 5: DISCUSSION

5.1 Log_{10} E. coli, MS2 and Turbidity Reduction Performance of Chitosan Acetate and Cloth Filtration

This study aimed to evaluate the effectiveness of a 10 mg/L of chitosan acetate dose for coagulation-flocculation pre-treatment, followed by cloth filtration to improve raw water quality. Three different stirring conditions were implemented to optimize the coagulation-flocculation and sedimentation procedures. Log_{10} Reduction Values (LRVs) were used to determine the effectiveness of the coagulant treatment and cloth filters to remove microbial colloidal contaminants and turbidity. The Mastersizer 3000 by Malvern was used to measure floc size formation during the coagulation-flocculation and sedimentation periods using the three stirring conditions. The two microorganisms, *E. coli* KO11 and MS2 coliphage, were chosen as the test microbes, and two different challenge waters were used: non- and 1% pasteurized sewage amended raw surface water from University Lake.

The chitosan acetate dose, 10 mg/L, was chosen based on preliminary jar test studies comparing four different chitosan acetate doses (0 mg/L, 10 mg/L, 20 mg/L, and 30 mg/L) under the same three stirring conditions used in the current study, in addition to a fourth condition with 1-minute of stirring at 100 rpm and a 24-hour sedimentation period. The standard and intermediate stirring conditions with 10 mg/L of chitosan acetate resulted in the lowest turbidity value, and the minimal stirring conditions, with the same 10 chitosan mg/L dose resulted in the highest turbidity value (**Appendix A**). Twelve layers of 100% cotton material was chosen for filtration based on previous work.

Filtration alone resulted in average LRVs ranging from 0.1 – 0.36 for test bacteria and viruses, and about a third of a Log_{10} reduction in turbidity (**Table 4.2**).

All average LRVs due to chitosan coagulation-flocculation pre-treatment and filtration for *E. coli* KO11 were >3, with intermediate stirring conditions in 1% sewage-amended challenge waters achieving an average LRV of 4.1±0.5. Bacterial reduction due to pre-treatment alone achieved just above 1 Log_{10} reduction with standard and intermediate stirring conditions in both challenge water types; however, minimal stirring conditions resulted in less than 1 LRV with chitosan coagulation-flocculation-sedimentation pre-treatment alone in both challenge water types (**Figure 4.1, Figure 4.2**).

Reductions of MS2 with both chitosan coagulation-flocculation pre-treatment and filtration resulted in LRVs greater than 3 for all stirring conditions and both challenge water types. Chitosan coagulation-flocculation-sedimentation pre-treatment alone was able to achieve greater than 1 LRV of MS2 with all stirring conditions in both challenge water types. Standard and intermediate stirring conditions were able to achieve average LRVs of 2.4±0.3 and 2.4±0.2 with pre-treatment alone, respectively.

E. coli KO11 and MS2 reductions due to cloth filtration and chitosan coagulation-flocculation-sedimentation pre-treatment were on average significantly more than 3 Log_{10} reductions greater than reductions due to filtration alone, with *p-values* < 0.00001. Turbidity reductions due to chitosan coagulation-flocculation-sedimentation pre-treatment with intermediate stirring conditions and cloth filtration resulted in the highest average LRVs of 1.4±0.03 for non-sewage amended samples and 1.2±0.2 for sewage amended samples. These reductions were on average, 0.98 LRVs more than reductions due to filtration alone with a *p-value* < 0.00001 (**Table 4.6**).

Performance targets for HWTS have been set by WHO to identify and classify the effectiveness of various technologies. Technologies that are deemed "protective" achieve a minimum of 2 Log_{10} reductions for bacteria and 3 Log_{10} reductions for viruses. Those technologies deemed as "highly protective" achieve a minimum of 4 and 5 Log_{10} reductions for bacteria and viruses, respectively (WHO, 2014b).

Average bacterial reductions of *E. coli* KO11, using the three stirring conditions in both challenge water types resulted in >3.0 Log_{10} reductions; intermediate stirring conditions produced significantly higher reductions in both water types. Average viral reductions for MS2, using the three stirring conditions in both challenge water types, resulted in >3.0 Log_{10} reductions; there was no significant difference in LRVs among the three stirring conditions. Microbial reduction results of this study meet WHO's HWTS International Scheme performance targets for *Protective* technologies (2-star tier) for both bacterial and viral reductions (WHO, 2016). Furthermore, raw turbidity results for effluent water after pre-treatment and filtration using intermediate and standard stirring conditions in both challenge water types was consistently < 1 NTU; this is consistent with turbidity guidance values set by WHO and with US EPA standards (WHO, 2011b).

In previous work using chitosan coagulation-flocculation and cloth filtration, a 35 mg/L dose of chitosan acetate was used. However, these results were not promising and achieved only slightly greater than 1 Log_{10} reductions (**Appendix B**). It was later determined that this chitosan dose was too high and jar tests, based on turbidity reduction and raw effluent turbidity values, identified a dose of 10 mg/L as a more effective in comparison to 20 mg/L and 30 mg/L of chitosan acetate.

As seen in **Figure 4.14**, the pore size of the cloth filter is ~100 μm. Typically, bacteria range from about one to five micrometers in diameter and viruses range from 25 to 90 nanometers

in diameter. While bacteria and viruses are not captured by basic cloth filtration, coagulating and flocculating these microbes with chitosan increased the overall sizes of suspended contaminant particles that can then be easily settled out or filtered.

The increased effectiveness of a lower chitosan dose of 10 mg/L is highlighted in an overall increase of LRVs under coagulation-flocculation pre-treatment conditions, with and without filtration. In previous work, when only the minimal stirring condition was employed, pre-treatment alone achieved average LRVs ranging from 0.21 – 0.51 for bacteria and 0.63 – 0.72 for viruses. In the current study, pre-treatment alone with 10 mg/L of chitosan acetate dose and minimal stirring conditions resulted in average LRVs ranging from 0.78 – 0.95 for bacteria and 1.3 – 1.8 for viruses. Additionally, raw turbidity values of effluent water from previous work was never <1 NTU, while effluent produced from the current study always resulted in raw turbidity values <1 NTU. This result is consistent with findings in another study that concluded chitosan doses > 10 mg/L to be less effective in turbidity removal (Soros, Amburgey, Stauber, Sobsey, & Casanova, 2019).

While reductions may be due to coagulation-flocculation-sedimentation procedures and physical removal due to filtration, reported LRVs may, in part, be attributable to direct aggregation or "clumping" of organisms that bind together, sometimes in flocs. This allows the overall effluent water microbial concentration to appear much lower than in actuality. Physical and chemical changes can be made to effluent waters that would reveal the true concentration of microbial contaminants in these samples. In the current study, 0.5 mL of sample effluent water was taken to create 10-fold serial dilutions for culture-based assay analysis. However, to account for aggregated microorganisms or microbes trapped in flocs in treated water, a larger sample of effluent water (~30-50 mL) could be collected and centrifuged to create a microbial pellet. The pellet could then be re-suspended in 10 mL of phosphate buffer for microbial analysis. In previous work, microbial

pellets have been suspended in 10 ml of microbial eluent (3% (w/v) beef extract at pH 9.5 + 0.1% Tween 80). This mixture acts as an emulsifier and surfactant to disperse aggregated or clumped microbes. Microbial concentrations could then be quantified by MPN methods with Colilert Quantitray for bacteria and DAL methods for bacteriophages. By centrifuging and re-suspending microbes from a larger sample volume, aggregated microbial particles may become dislodged from each other, allowing for a more accurate portrayal of the microbial water quality. This outcome could also be achieved by altering the chemical composition of effluent waters. Decreasing the pH of treated water causes suspended flocs or aggregated microbes to disperse (Soros, 2015).

Floc sizes formed after treatment with a 10 mg/L dose of chitosan acetate in non-sewage amended raw surface waters sampled in August were significantly larger than flocs formed from the addition of 10 mg/L dose of chitosan acetate to raw water sampled from March and amended with 1% pasteurized sewage (**Table 4.8**).

A case study conducted in Bangladesh evaluated the effectiveness of simple saree cloth filtration in reducing *V. cholerae* particles in filtered surface water sources and the magnitude of hospital visits due to cholera related illnesses. A 2 Log_{10} reduction in *V. cholerae* was achieved with the use of the saree cloth filter and a 48% decrease in hospital cases was associated with filtration. While the "highly protective" target set by the WHO's HWTS scheme was not met with combined use of chitosan and cloth filtration, the "protective" performance target was met and, therefore, it is likely that adverse health effects can still be reduced by applying chitosan coagulation-flocculation-sedimentation followed by cloth filtration.

The particle size analysis resulted in measured floc sizes over the coagulation-flocculation and sedimentation period. Standard stirring procedures produced flocs that were significantly larger than those created by minimal and intermediate stirring conditions for both challenge waters

(*p < 0.000010).* Flocs produced by all three stirring conditions in water sampled in August were significantly larger than flocs produced with all three stirring conditions in water sampled in March. This difference is speculated to be a result of the variation in water quality among the two challenge waters used, specifically differences in TOC, alkalinity, turbidity, and conductivity.

While Dx(50) measurements for chitosan flocs formed in August 2018 sampled waters were generally greater than the measured pore size of the cloth filters, the Dx(10) measurements, or smallest 10% of particles, were smaller than the cloth filter pore size. These flocs that are smaller than the measured pore size, along with pathogens and particles that are not captured by the chitosan floc, would likely readily pass through the filter without being removed. Turbidity was reduced by >90% after pre-treatment and filtration (**Table 4.5**). It is plausible that the remaining particles that contributed to effluent turbidity may be a result of the smallest 10% of particles that were not physically removed by the cloth filters after pre-treatment. Because the larger floc particles are likely to contain a lot more microbes than the smaller floc particles, the reductions in microbes could potentially be greater than the actual reductions in physical floc particles quantified individual units in the particle size analyzer.

While intermediate stirring conditions resulted in significantly higher LRVs for *E. coli* and higher, but non-significant, LRVs for MS2, floc size from particle size analysis indicated smaller floc size formation with the use of the intermediate stirring condition. This inconsistency in results may have been due to the limitation in the apparatus set-up of the Mastersizer analyzer. Challenge water was pushed through the Mastersizer using a peristaltic pump, which was required to collect the samples. The pumping action caused a regular physical disturbance in the water, similar to the motion of mixing the water. Pumping and the transfer in and out of the tubing may have caused fragmentation of the formed flocs, resulting in measured floc sizes that were much lower than

actual floc sizes for all experiments. Each stirring procedure was associated with varying mixing and settling times; therefore, floc formation during each stirring procedure may have been affected differently. Although particle size analysis may not have revealed true floc size formation in microbial experiments, relative floc sizes determined among challenge water types and stirring conditions may still be useful information. Water collected in March had a lower a total organic content and alkalinity than water sampled in August—due to seasonal changes—and also produced smaller flocs (**Table 4.7**).

Average MS2 LRVs due to chitosan acetate coagulation-flocculation pre-treatment alone were, on average, 0.72 higher than *E. coli* KO11 LRVs; this was a significant finding with a *p-value* < 0.000010. This may be explained by the difference in isoelectric points between *E. coli* KO11 and MS2. An isoelectric point (pI) is the pH value at which a particle or molecule has a net-neutral charge in solution (Moldoveanu & David, 2013). The isoelectric point of *E. coli* KO11 is 5.6, whereas MS2 has an isoelectric point of 3.5-3.9. This indicates that MS2 coliphage, which has a negative charged surface, is rendered neutrally charged at a pH of 3.5 to 3.9, whereas *E. coli* KO11 may be rendered neutrally charged at a higher pH level around 5.6 (Collins et al., 2006; Sherbet & Lakshmi, 1973). The average pH of water used for microbial experiments was 7.6±0.29 (**Table 4.1**). Because MS2 has a lower isoelectric point, it has a larger negative charge in the test water near pH neutral, as compared to *E. coli* KO11. Due to this difference in magnitude of surface charge, MS2 may have had a stronger electrostatic attraction with the positively-charged chitosan acetate coagulant, resulting in a higher LRV from chitosan coagulation-flocculation pre-treatment alone. Another reason for the difference in LRVs between MS2 coliphage and *E. coli* KO11 may have been due to variations in the surface-area-to-mass-ratio. Smaller suspended particles have a higher surface-area-to-mass-ratio, whereas larger particles have a lower-surface area-to-mass-

ratios. Particles suspended in water, including bacteria and viruses, typically have a negative surface charge. The greater the particle's surface area relative to the particle's mass, the greater the effect of the charge on the particle's movement and interaction with other compounds. MS2 coliphage measures ~27 nm while *E. coli* KO11 measures ~2000 nm. Because MS2 has a larger surface-area-to-mass-ratio, the negative surface charge may play a larger role in its mobility and interaction with other particles and compounds, likely making MS2 more effectively removable with chitosan coagulation, as compared to *E. coli* KO11 (Engelhardt, 2010).

Hydrophobic interactions, in contrast to electrostatic and ionic interactions, are driven by the interactions between nonpolar surfaces. While chitosan has a net positive charge when dissolved in water, the linear polysaccharide is actually amphiphilic in nature, allowing hydrophobic interactions with viruses. Chitosan is comprised of neutral and positively charged sugar units. The neutral units (GlcNAc), or A-units, are responsible for the hydrophobic effects and contain a rather bulky acetyl group. The charged units (GlcN), or D-units, are responsible for the hydrophilic/electrostatic interactions and contain a positively charged amino group ($-NH_3^+$) produced by a deacetylation and protonation process described in **Figure 2.1**. Chitosan's hydrophobicity depends on the chain length and the ratio of A-units to D-units (F_A), which depends on the DD (Nilsen-Nygaard et al., 2015). Virus surfaces often have both hydrophobic and hydrophilic surfaces with some viral surfaces being more hydrophobic than others. For instances, the surface of MS2 coliphage has more polar characteristics as compared to that of Q-beta which has more apolar characteristics and is therefore more responsive to hydrophobic interactions (Armanious et al., 2016). Hydrophobic effects play a role in the coagulation process when there is viral adsorption to hydrophobic acetyl regions (A-units) of the chitosan chain. As amino groups on the D-units deprotonate at higher pH levels, hydrophobic interactions become more dominant

as compared to electrostatic and ionic forces (Nilsen-Nygaard et al., 2015; S. P. Strand et al., 2001; Sabina P. Strand et al., 2003). Natural waters with negatively charged particles such as bacteria, viruses, protozoans, clay, and silt, typically have a pH ranging from 5 to 9 (Crittenden & Montgomery Watson Harza (Firm), 2012); hydrophobic effects of chitosan may play a greater role in more basic natural waters with higher pH. E.M. van Voorthuizen et al. (2001) found that hydrophobic interactions play an important role in the adsorption and retention of MS2 coliphage on filters. There is also evidence suggesting that above the pI of a particular virus, hydrophobic effects may be more important for adsorption than previously thought (Van Voorthuizen, Ashbolt, & Schäfer, 2001). While the effects of hydrophobicity on viral adsorption and coagulation-flocculation were not studied in the current work, it is one possible explanation for the higher observed MS2 coliphage LRVs.

There was no significant difference between reductions in MS2 and reductions in *E. coli* KO11 due to cloth filtration and chitosan coagulation-flocculation-sedimentation pre-treatment together. The explanation for significantly higher LRVs in MS2 in chitosan coagulated-flocculated pre-treated samples are speculative and would require further studies to draw definitive conclusions regarding LRV difference among microbes due to electro-kinetic properties. Because coagulation may be more effective with microbes that have a lower pI or larger diameter, it is important to test the effectiveness of coagulation pre-treatment and filtration with microbes that have a range of characteristics. Coliphage MS2, with a diameter of ~27 nm and a pI of 3.5-3.9, is smaller in size as compared to many other viruses that range from 20 nm – 85 μm in size. Bacteriophage Q-beta has a higher pI of 5.3 but is similar in diameter to MS2, reported as approximately 26 nm. Bacteriophage PRD1 has a pI of 4.2 and diameter of ~66 nm. These

microbes could be used to evaluate the effects of bacteriophage pI and size on coagulation and filtration processes (Dowd, Pillai, Wang, & Corapcioglu, 1998; Lim, Spingola, & Peabody, 1996).

This research aimed to evaluate the use of chitosan coagulant as an alternative to traditional inorganic coagulants. Chitosan is an attractive alternative coagulant option due to its biodegradability, relative abundance, non-toxicity, and overall sustainability. Since pH and coagulant dose are not easily optimized in household settings, inorganic coagulants are not the best option. Chitosan has been tested with various filters and shows effectiveness over a range of doses and does not drastically alter the pH of water (Renault, Sancey, Badot, & Crini, 2008). Chitosan jar tests were able to achieve 3-5 Log_{10} reductions for MS2 and *E. coli* over a range of doses (Soros et al., 2015). Additionally, studies have shown the ability of chitosan to be an effective coagulant in combination with other filtration technologies such as CWFs (Abebe et al., 2016).

5.2 Chitosan Mechanisms

The current study did not directly study mechanisms by which chitosan removed viral, bacterial, and other colloidal particles from challenge waters. However, literature has provided insight about plausible mechanisms by which chitosan operates, although explanations are only informed speculation. Charge neutralization and inter-particle bridging are the two major mechanisms by which chitosan is speculated to work. Charge neutralization occurs when negatively charged particles such as viruses, bacteria, silt, clay, and other organic and inorganic matter, adsorb onto positively charged sites of the chitosan polymeric chain. Coagulation-flocculation processes are promoted as this mechanism occurs repeatedly. Flocs that are large and dense enough will sediment out due to gravity, reducing viral, bacterial, and colloidal particles in the supernatant water. While *E. coli* KO11 is volumetrically larger than MS2, and therefore more readily filtered out, MS2 has a lower isoelectric point and therefore possesses a greater negative

charge in water near neutral pH. These mechanisms are mentioned in available literature, but the physio-chemical interactions between chitosan flocs and cloth filters are not well described (M. N. V. Ravi Kumar et al., 2004; Rinaudo, 2006; Soros et al., 2019, 2015).

5.3 Prior Work

The saree-cloth filtration field study conducted in rural Bangladeshi communities found that 4-8 layers of saree cloth were able to achieve 2 Log_{10} reductions in *V. cholerae.* The cholera bacterial particles had a preferential attachment to copepods found naturally in the Bangladeshi water sources. The copepods, with a diameter of a few millimeters, were readily filtered out, and therefore reduced the number of cholera particles in the water. This study also found that worn and older saree cloth resulted in a smaller overall pore size, as compared to new saree cloth, due to agitation and fraying of individual fibers through repeated use. (Colwell et al., 2002). In the current study, twelve layers of new, 100% cotton linen cloth were used as a filter. The same four filters were used for all microbial experiments, but they were not scrubbed with the intention to disrupt the fabric threads or to create a specific pore size. The cloth filters were washed in a chlorine solution, autoclaved, and air dried. Filter pore size can be observed in **Figure 4.14**. Had the cloth been agitated further to promote fraying and other physical alteration, LRVs may have been larger than values observed in the current study. However, LRV with cloth usage over time was not evaluated as an experimental variable, and these impacts on microbial reduction performance are purely speculative.

Although the use of *M. oleifera* as an organic coagulant is able to achieve some level of bacterial reduction, chitosan acetate at the recommended dose is able to achieve > 3-Log_{10} reductions of both *E. coli* KO11 bacteria and MS2 coliphage, achieving the 2-star performance

level for HWTS set by WHO and was also able to reduce turbidity < 1 NTU in challenge waters used for microbial analysis.

While this study did not evaluate cloth filtration for its effectiveness at removing bacteria spores, protozoans, or other microorganisms, the relevant literature suggests cloth filtration may be suitable for the removal of larger pathogens through size exclusion. Aside from viruses and bacteria, which are on the order of nano- and micro-meters in size, respectively, many water-associated and waterborne pathogens such as protozoan parasites are comparatively large in size, with many >10 µM in size (Sobsey, 2002). Larvae within intermediate crustacean hosts, and pathogens associated with larger zooplankton or copepods (such as *V. cholerae*) may be readily filtered out using cloth filtration. Paper, nylon, and polyester filters are often recommended for removal of schistosomes and the *Cyclops* vector of guinea worm (Imtiaz, Anderson, Long, Sullivan, & Cline, 1990). Studies have shown the effectiveness of these cloth filters at community and household levels (Aikhomu, Brieger, & Kale, 2000). While recommended for larger organisms or bacteria that attach onto larger organisms, cloth filtration alone is not recommended for the removal of bacterial and viral particles, and therefore, a multi-barrier approach for HWTS employing cloth is required.

The aim for this research was to evaluate the potential to improve microbial reductions with the use of chitosan coagulation-flocculation as a pre-treatment process prior to cloth filtration. The WHO HWTS performance tiers for bacterial and viral reductions were used and are based on acceptable risk or tolerable disease burden defined by health-based targets presented in DALYs. Chitosan acetate, at a 10 mg/L dose, with the quality of water detailed in **Table 4.1**, was able to achieve the WHO 2-star protective performance level (>3-Log_{10} reductions for viruses and >2-Log_{10} reductions for bacteria). This was significantly better than cloth filtration alone, which was

unable to reach even the WHO 1-star level. The use of chitosan acetate at the suggested dose, as a pre-treatment coagulation-flocculation process prior to filtration with 12-layers of cotton cloth provides substantial reductions in bacteria and viruses, therefore, likely reducing morbidity risks in comparison to filtration through these cloth filters alone.

While chitosan coagulation-flocculation pre-treatment partnered with cloth filtration may not be as effective at removing microbial contaminants as chitosan pre-treatment partnered with sand filters or CWFs, the treatment combination was able to achieve the 2-star protective performance target of WHO. In communities where CWFs or sand filters are already utilized, cloth filtration may not be a preferred recommendation for filtration treatment because more robust filtration technologies are already implemented. However, chitosan pre-treatment may be well partnered with CWFs and sand filters to improve the effectiveness of these filtration technologies to reduce microbial contaminants and turbidity. Where cloth/saree filters are already commonly used, chitosan pre-treatment could be implemented. Cloth filters are an attractive and cost effective filtration option because filtration time is relatively low and filters are made of scrap cloth that is already available from the household; hence, there is no need to purchase a new technology. Furthermore, unlike CWFs, cloth filters do not require specific water collection containers , they cannot crack like CWFs, they do not require the periodic scouring as is required of CWFs or BioSand filters to restore flow rates and the cloth filtration apparatus can be adjusted to the water filtration preferences of the household.

While field implementation is the ultimate goal for any POU/HWTS, it was not a goal of this study. It was beyond the scope of this project to delineate the steps required for subsequent field implementation of this treatment. Instead, we conducted a proof-of-concept lab study based on microbial reduction performance of POU technology.

5.4 Limitations

This research served to evaluate the effectiveness of the chitosan acetate coagulation-flocculation and sedimentation process as a pre-treatment in combination with 12-layers of 100% cotton cloth filtration, with a focus on the reduction of bacteria, viruses, and turbidity. However, there are limitations that should be mentioned and addressed in future studies.

While cloth filtration with chitosan pre-treatment was found to achieve the 2-star level of protection for bacteria and viruses as specified by the WHO HWTS performance targets, the removal of protozoa was not specifically studied. An assumption was made that because protozoa are larger in size when compared to bacteria and viruses, that protozoan LRVs due to chitosan coagulation-flocculation and cloth filtration would be similar to if not greater than LRVs achieved for bacteria or viruses. Furthermore, the study did not distinguish between the formation of flocs containing microbial contaminants due to the chitosan coagulation-flocculation process and sedimentation prior to filtration and the direct aggregation and clumping of microbes in the absence of chitosan coagulation-flocculation treatment.

The effects of water quality on the effectiveness of chitosan as a coagulant was not exhaustively or systematically studied. A convenient and representative sample water was used for all microbial experiments, and two different samplings of water were used for particle size analysis of flocs with the Mastersizer 3000 by Malvern. Water sampled in March 2019, with a lower alkalinity and TOC, gave significantly lower floc size formation among all three stirring conditions compared to the water collected at a different time of the year. While water quality was not directly studied, it is speculated that water quality parameters, such as alkalinity and TOC, may have an effect on chitosan's ability to form larger flocs. Seasonality and temperature of challenge water were not studied but may impact chitosan acetate coagulation-flocculation and filtration performance.

In addition to laboratory and apparatus limitations, there are also limitations to extrapolating these findings to the use of this technology in the field. Firstly, each cloth was decontaminated between experiments using a bleach solution and autoclaved to prevent microbial re-growth. However, this is unlikely to be an accessible form of decontamination in the field. The filters would likely be cleaned simply with water and a small amount of soap. Re-growth of accumulated microbes on the filters with a minimal cleaning procedure is possible but no conclusive determination about its likelihood can be made on the basis of this research. Additionally, new cloth filters were used for the first set of experiments, and the same filters were used subsequently for every experiment. Studies have shown the improved effectiveness of old/used cloth over new cloth filters (Colwell et al., 2002). Change in LRVs due to repeated filter use was not assessed. Furthermore, the practicality, acceptance and sustained use of intermediate stirring conditions among communities and users would need to be studied, likely in a human-centered design study.

The Mastersizer 3000 by Malvern was employed to measure floc size formation over the time period of the coagulation-flocculation and sedimentation process. While this equipment was able to provide readings of the floc particle sizes as they passed in front of the cell, the peristaltic pump used to push the coagulating water through the machine created disturbance and agitation in the water. This imitated a continuous stirring motion in the water and likely fragmented flocs during such motions within the equipment tubing. This resulted in 1) floc size readings that were much smaller than flocs observed in microbial experiments (which occurred without the peristaltic pump and Mastersizer) and 2) no true, undisturbed sedimentation period for the coagulated water during the floc size analysis experiments. While these limitations should be taken into consideration when using the data to speculate on chitosan's true ability to form flocs of a specific

size, the data can still be used to objectively compare 1) the various stirring conditions and 2) the effects of two different water qualities on the ability of 10 mg/L dose of chitosan acetate to form flocs. Variability in influent water characteristics also had an influence on chitosan floc formation, however, the effects of a singular water quality parameter on floc formation were not isolated and studied for this project.

5.5 Future Work

Future work in this field could include looking at the effects of different cloth layers with this identified optimal dose of 10 mg/L of chitosan acetate. Additionally, different cloth material, such as engineered cloth or paper filters could also be evaluated. Many engineered cloth materials have pore sizes < 1 μm; the use of these materials with chitosan pre-treatment could result in LRVs greater than the LRVs established in the current study. However, there is the potential for flocs to clog the pores of filters with smaller pore sizes. The effectiveness of the cloth filters after consistent and repeated use could also be studied since used and worn-out cloth filters have been shown to have a smaller effective pore size (Colwell et al., 2002). A longitudinal study with the cloth filters with consistent dosing could be conducted to assess the prolonged effectiveness of the filters. Additionally, various cleaning methods of the filters could be employed to test for potential microbial re-growth and other impacts on microbial reduction performance.

Various water qualities with different levels of alkalinity, pH, TOC, conductivity, turbidity and temperature, should be tested to better understand the effectiveness of chitosan acetate coagulation-flocculation to address the seasonal differences in the quality of natural waters. Natural surface water from only University Lake located in Chapel Hill, Orange County, NC was sampled and used for all experiments. Water used for microbial experiments had an average

turbidity level of 6.21±0.72; however, challenge waters with higher and lower turbidities could be used in future studies.

Different filtration devices may also be a direction to further explore, including CWFs, BioSand filters, or activated carbon filters. Longitudinal studies with CWFs have been explored but have encountered obstacles due to filter pore clogging. A potential remedy to consider is a 1-minute rapid stir followed by a 24-hour settling period. This settling period was able to produce supernatant water with a turbidity well below 1 NTU, as seen in **Appendix A**. The coagulated flocs could also be filtered through cloth prior to filtration through CWFs.

CHAPTER 6: SUMMARY AND CONCLUSIONS

This research aimed to evaluate the use of a 10 mg/L dose of chitosan acetate as a coagulation-flocculation-sedimentation pre-treatment process prior to filtration through 12 layers of 100% cotton cloth material on the basis of the reductions of *E. coli* KO11, MS2, and turbidity. Three different stirring conditions during flocculation were utilized to identify optimal coagulation-flocculation and sedimentation procedures while remaining mindful of reasonable user practices. Two different challenge waters were used: non-amended and 1% pasteurized sewage-amended surface waters. A particle size analyzer, the Mastersizer 3000 by Malvern, was used to evaluate floc size formation during the three stirring conditions. While particle size analysis resulted in the standard stirring conditions producing the largest average sized flocs for both challenge waters, intermediate stirring conditions resulted in *E. coli* KO11 reductions that were significantly higher than standard and minimal stirring conditions. Intermediate stirring conditions also resulted in higher reductions in MS2 and turbidity, but this was not always a significant difference in reduction. Reductions in MS2 coliphage due to pre-treatment by chitosan acetate coagulation-flocculation alone were statically significant. All three stirring conditions resulted in $> 3\text{-Log}_{10}$ reductions for both *E. coli* KO11 and MS2 coliphage, which meets the WHO protective performance target (2-star level) for reductions of bacteria and viruses set by WHO for HWT technologies. Turbidity was also reduced by $> 1\text{-Log}_{10}$. Turbidity of effluent waters after pre-treatment and filtration were always reduced to < 1 NTU, which meets EPA standards and guidance by WHO. Furthermore, pH was not significantly altered by chitosan coagulation-flocculation treatment.

The effects of seasonality, alkalinity, conductivity, temperature, pH, TOC, turbidity and overall water quality were not directly studied in this research. With the consideration of the expressed limitations and need for future work, this research study demonstrates the ability of chitosan acetate coagulation-flocculation and sedimentation to significantly improve the microbial reduction performance of cloth filtration as a point-of-use water treatment technology. Further optimization of this technology should be considered in future work.

APPENDIX A: PRELIMINARY EXPERIMENTS

Method #1: Intermediate stirring

- 1-minute rapid stirring
- 3x [2-minutes stir & 5-minutes rest]
- 25-minute settle

Method #2: Standard

- 1-minute @ 100rpm
- 15-minutes @ 25 rpm
- 30-minutes settling

Method #3 24-hour settling period

- 1-minute rapid stirring
- 24-hours settling

Method #4: Original

- 1-minute rapid stirring
- 30-minutes settling

Table A.1 Turbidity (NTU) was measured and recorded for various doses of chitosan acetate (10 mg/L, 20 mg/L, 30 mg/L) and coagulation-flocculation-settling procedures. Experiments were performed in triplicates. Test water contained 1% pasteurized sewage.

Method #1: Intermediate stirring							
	Control (0mg/L)	10 mg/L	%Δ (10 mg/L)	20 mg/L	%Δ (20 mg/L)	30 mg/L	%Δ (30 mg/L)
	7.35	1.63	-77.82	4.23	-42.45	7.15	-2.72
	7.12	1.73	-75.70	4.16	-41.57	7.50	5.34
	7.14	1.52	-78.71	4.18	-41.46	7.38	3.36
	6.43	1.94	-69.83				
	6.43	2.62	-59.25				
Average	6.89	1.89	-77.41	4.19	-41.83	7.34	1.99
Standard Error	0.19	0.20	0.89	0.02	0.31	0.10	2.42
Method :2: Standard Coagulation and Flocculation Procedure							
	Control (0mg/L)	10 mg/L	%Δ (10 mg/L)	20 mg/L	%Δ (20 mg/L)	30 mg/L	%Δ (30 mg/L)
	6.55	1.74	-73.44	6.09	-7.02	5.83	-10.99
	5.93	2.32	-60.88	4.57	-22.93	6.64	11.97
	5.94	2.00	-66.33	4.94	-16.84	6.55	10.27
	6.50	1.60	-75.38				
	5.83	1.60	-72.56				
Average	6.15	1.85	-66.88	5.20	-15.60	6.34	3.75
Standard Error	0.15	0.14	3.64	0.46	4.63	0.26	7.39
Method #3: 24-hour settling period							
	Control (0mg/L)	10 mg/L	%Δ (10 mg/L)	20 mg/L	%Δ (20 mg/L)	30 mg/L	%Δ (30 mg/L)
	6.11	0.704	-88.48	1.25	-79.54	2.59	-57.61
	6.40	0.652	-89.81	1.27	-80.16	2.22	-65.31
	6.55	0.480	-92.67	1.42	-78.32	2.52	-61.53
	5.99	0.388	-93.52				
	6.08	0.482	-92.07				
Average	6.23	0.54	-90.32	1.31	-79.34	2.44	-61.48
Standard Error	0.11	0.06	1.24	0.05	0.54	0.11	2.22
Method #4: 1 minute stir- 30 minute settle							
	Control (0mg/L)	10 mg/L	%Δ (10 mg/L)	20 mg/L	%Δ (20 mg/L)	30 mg/L	%Δ (30 mg/L)
	6.11	8.78	43.70	8.06	31.91	8.01	31.10
	6.40	8.71	36.09	8.31	29.84	7.98	24.69
	6.55	8.97	36.95	8.36	27.63	7.93	21.07
Average	6.35	8.82	38.91	8.24	29.80	7.97	25.62
Standard Error	0.13	0.08	2.41	0.09	1.24	0.02	2.93

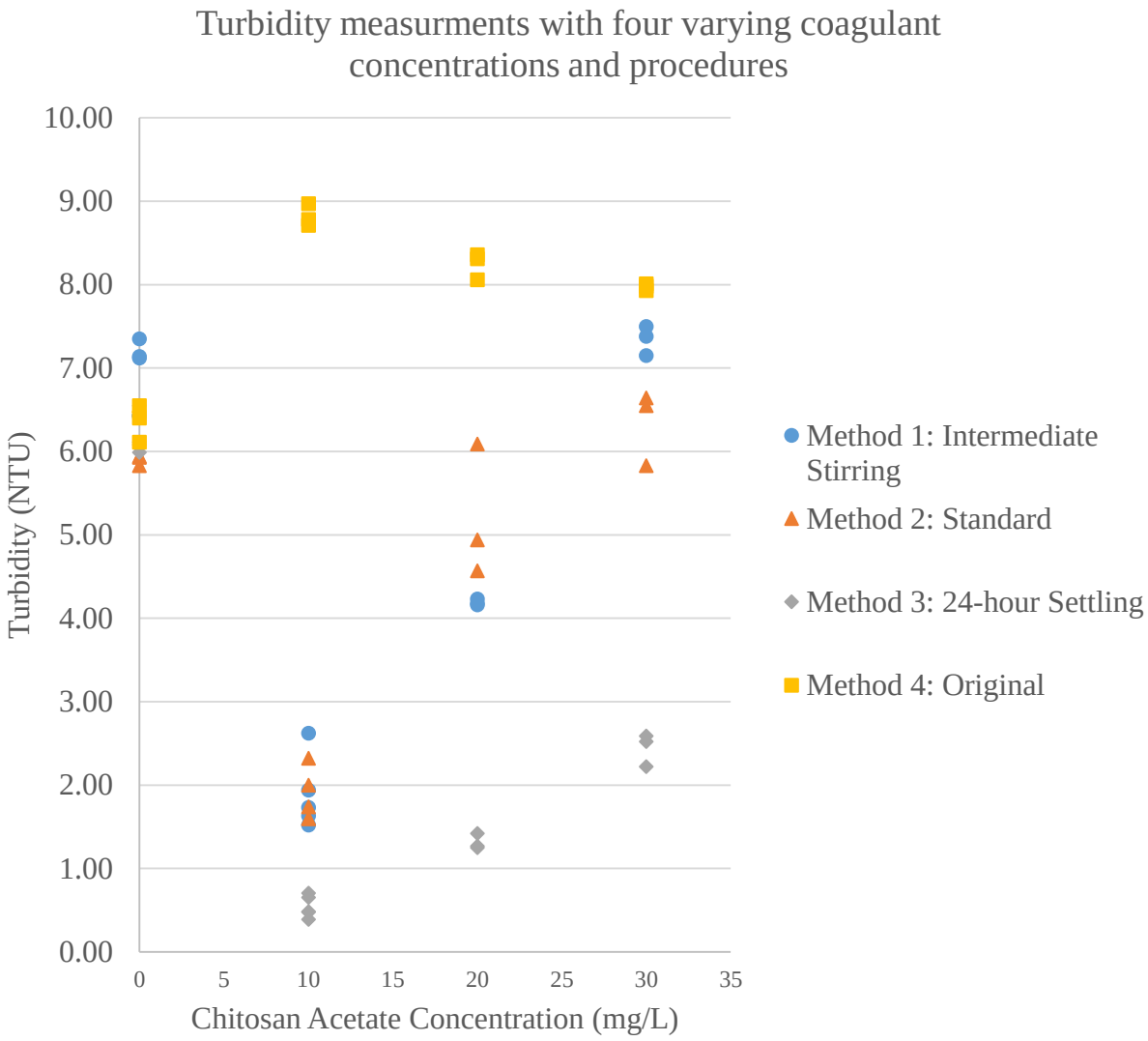

Figure A.2 Displays the same data as in **Table A.1** but with raw data points instead of percent change.

APPENDIX B: PREVIOUS WORK

	No Chitosan; 12-Layers	35mg/L Chitosan Pre-filter	Post-Chitosan to Effluent	Influent to Effluent
MS2 (No Sewage)	0.3 ± 0.2	0.7 ± 0.06	0.6 ± 0.04	1.2 ± 0.2
MS2 (Sewage)	0.1 ± 0.01	0.6 ± 0.1	0.6 ± 0.1	1.3 ± 0.09
E. coli B (No Sewage)	0.3 ± 0.03	0.4 ± 0.1	0.9 ± 0.2	1.4 ± 0.1
E. coli B (Sewage)	0.2 ± 0.07	0.4 ± 0.1	0.4 ± 0.1	0.9 ± 0.3
V. cholerae (No Sewage)	0.1 ± 0.06	0.3 ± 0.2	0.6 ± 0.3	1.3 ± 0.3
V. cholerae (Sewage)	0.2 ± 0.07	0.5 ± 0.1	0.8 ±30.1	1.08 ± 0.1
E. coli KO11 (No Sewage)	0.4 ± 0.06	0.3 ± 0.06	0.4 ± 0.06	0.6 ± 0.06
E. coli KO11 (Sewage)	0.3 ± 0.02	0.2 ± 0.06	1.3 ± 0.3	1.2 ± 0.3

Figure B.1 Log_{10} reductions for MS2 coliphage, *E. coli* B, *V. cholera*, and *E. coli* KO11 for 1%-pasteurized sewage and non-pasteurized sewage amended water samples with 35 mg/L dose of chitosan acetate.

Table B.1: Raw data of turbidity (NTU) and pH of *E. coli* B and MS2 bacteriophage samples with 35 mg/L dose of chitosan acetate pre-treatment followed by filtration through 12 layers of 100% cotton T-shirts including average percent changes, sample size, and calculated standard error.

Past. Sewage	No									
Parameter	pH					Turbidity (NTU)				
Sampling Point	IN	CH	EF	% Δ (CH-EF)	% Δ (IN-EF)	IN	CH	EF	% Δ (CH-EF)	% Δ (IN-EF)
	7.50	6.83	6.98	2.20	-6.93	7.91	7.91	3.11	-60.68	-60.68
	7.50	6.83	6.90	1.02	-8.00	7.91	7.91	3.06	-61.31	-61.31
	7.60	6.50	6.95	6.92	-8.55	7.85	7.97	3.56	-55.33	-54.65
Average	7.53	6.72	6.94	3.38	-7.83	7.89	7.93	3.24	-59.11	-58.88
Sample Size	3	3	3	3	3	3	3	3	3	3
Standard Error	0.03	0.11	0.02	1.80	0.48	0.02	0.02	0.16	1.90	2.12

Past. Sewage	yes									
Parameter	pH					Turbidity (NTU)				
Sampling Point	IN	CH	EF	% Δ (CH-EF)	% Δ (IN-EF)	IN	CH	EF	% Δ (CH-EF)	% Δ (IN-EF)
	7.40	6.85	6.95	1.46	-6.08	8.81	9.16	4.31	-52.95	-51.08
	7.40	6.85	6.99	2.04	-5.54	8.81	9.16	5.26	-42.58	-40.30
	7.60	6.75	6.82	1.04	-10.26	9.04	9.23	4.93	-46.59	-45.46
Average	7.47	6.82	6.92	1.51	-7.29	8.89	9.18	4.83	-47.37	-45.61
Sample Size	3	3	3	3	3	3	3	3	3	3
Standard Error	0.07	0.03	0.05	0.29	1.49	0.08	0.02	0.28	3.02	3.11

Table B.2: Raw data of turbidity (NTU) and pH of *E. coli* KO11 and *V. cholerae* samples with 35 mg/L of chitosan acetate pre-treatment followed by filtration through 12 layers of 100% cotton T-shirts including average percent changes, sample size, and calculated standard error.

Past. Sewage	no									
Parameter	pH					Turbidity (NTU)				
Sampling Point	IN	CH	EF	% Δ (CH-EF)	% Δ (IN-EF)	IN	CH	EF	% Δ (CH-EF)	% Δ (IN-EF)
	7.95	8.01	7.09	-11.49	10.82	6.97	7.02	1.64	-76.64	76.47
	7.95	8.01	7.01	-12.48	11.82	6.97	7.02	1.27	-81.91	81.78
	7.95	8.01	7.05	-11.99	11.32	6.97	7.02	1.39	-80.20	80.06
Average	7.95	8.01	7.05	-11.99	11.32	6.97	7.02	1.43	-79.58	79.44
Sample Size	3	3	3	3	3	3	3	3	3	3
Standard Error	0.00	0.00	0.023	0.29	0.29	0.00	0.00	0.11	1.55	1.56

Past. Sewage	yes									
Parameter	pH					Turbidity (NTU)				
Sampling Point	IN	CH	EF	% Δ (CH-EF)	% Δ (IN-EF)	IN	CH	EF	% Δ (CH-EF)	% Δ (IN-EF)
	8.29	7.97	7.56	-5.14	-8.81	8.58	8.67	3.37	-61.13	60.72
	8.29	7.97	7.43	-6.78	10.37	8.58	8.67	3.12	-64.01	63.64
	8.29	7.97	7.44	-6.65	10.25	8.58	8.67	3.19	-63.21	62.82
Average	8.29	7.97	7.48	-6.19	-9.81	8.58	8.67	3.23	-62.78	62.39
Sample Size	3	3	3	3	3	3	3	3	3	3
Standard Error	0.00	0.00	0.042	0.52	0.50	0.00	0.00	0.074	0.86	0.87

APPENDIX C: CERTIFICATE OF ANALYSIS FOR FOOD GRADE CHITOSAN ACETATE

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CERTIFICATE OF ANALYSIS

Chemical Name	Chitosan Acetate		**PRODUCT #:**	S1247	
CAS No:	9012-76-4	**Manufacture Date:**	Dec. 28, 2017	**Report Date:** Dec. 29, 2017	**Exp. Date:** Dec. 28, 2019
Batch #:	**SL-3308**				

	Items	Specifications	Results
Product Properties	Deacetylation	≥85%	90.3%
	Appearance	White or light yellow powder	Complies
	Odor and taste	Characteristic	Complies
	pH	3-6	4.2
	Viscosity cps	10-100	17
	Insolubles%	≤1	0.3
	Density g/ml	0.28	0.302
	Ash%	≤1	0.72
	Moisture%	≤10	8.7
	Mesh Size mesh	80 (95% pass)	Complies
	Heavy Metals ppm	≤10	Complies
	As ppm	≤0.5	Complies
	Total plate count cfu/g	≤100	Complies
	Yeast & Mold cfu/g	≤10	Complies
	E.Coli cfu/g	Negative	Negative
	Salmonella cfu/g	Negative	Negative

Approved By:	S. Kumar	02.02.18

Any claims, adjustments or returns must be made within 30 days.

APPENDIX D: RAW LRVS FOR *E. coli* KO11 AND MS2 COLIPHAGE

E. coli KO11						Average	n	95% CI
No Sewage	Filtration Alone		0.124	0.093	0.075	0.098	3	0.028
	Standard	Post CH	1.241	0.959	1.426	1.209	3	0.267
		CH to EF	2.099	1.942	1.555	1.865	3	0.317
		Effluent	3.340	3.183	2.796	3.106	3	0.317
	Intermediate	Post CH	1.165	1.378	0.981	1.175	3	0.225
		CH to EF	2.108	3.187	1.932	2.409	3	0.769
		Effluent	3.273	4.353	3.097	3.574	3	0.769
	Original	Post CH	0.836	0.930	0.586	0.784	3	0.201
		CH to EF	2.472	1.848	2.659	2.327	3	0.481
		Effluent	3.309	2.684	3.496	3.163	3	0.481
Sewage	E0		0.028	0.229	0.104	0.120	3	0.115
	Standard	Post CH	1.359	1.513	0.937	1.270	3	0.337
		CH to EF	2.269	2.116	1.821	2.069	3	0.257
		Effluent	3.628	3.475	3.181	3.428	3	0.257
	Intermediate	Post CH	1.549	1.122	1.409	1.360	3	0.247
		CH to EF	2.060	2.935	2.634	2.543	3	0.503
		Effluent	3.610	4.485	4.184	4.093	3	0.503
	Original	Post CH	0.914	0.828	1.062	0.935	3	0.134
		CH to EF	2.429	1.724	2.690	2.281	3	0.565
		Effluent	3.344	2.638	3.604	3.195	3	0.565
MS2 Bacteriophage						Average	n	95% CI
No Sewage	Filtration Alone		0.099	0.133	0.081	0.104	3	0.030
	Standard	Post CH	1.833	1.398	1.814	1.682	3	0.278
		CH to EF	1.934	0.849	1.380	1.388	3	0.614
		Effluent	3.767	2.682	3.213	3.220	3	0.614
	Intermediate	Post CH	1.674	1.598	1.338	1.537	3	0.200
		CH to EF	1.707	1.697	2.220	1.875	3	0.338
		Effluent	3.382	3.294	3.558	3.411	3	0.152
	Original	Post CH	1.979	1.921	1.405	1.769	3	0.358
		CH to EF	1.276	0.808	1.427	1.170	3	0.365
		Effluent	3.255	2.787	3.406	3.150	3	0.365
Sewage	E0		0.423	0.562	0.079	0.355	3	0.282
	Standard	Post CH	2.396	2.599	2.081	2.359	3	0.295
		CH to EF	1.273	1.606	1.132	1.337	3	0.275
		Effluent	3.669	4.003	3.529	3.734	3	0.275
	Intermediate	Post CH	2.494	2.195	2.605	2.431	3	0.240
		CH to EF	1.002	1.441	0.983	1.142	3	0.294
		Effluent	3.496	3.935	3.477	3.636	3	0.294
	Original	Post CH	1.146	1.531	1.160	1.279	3	0.247
		CH to EF	2.368	2.589	2.028	2.328	3	0.320
		Effluent	3.514	3.734	3.173	3.474	3	0.320

APPENDIX E: RAW LOG_{10} REDUCTIONS FOR TURBIDITY AND PH

Challenge Water	Method		pH % Δ	Turbidity % Δ	Turbidity Log_{10} Reduction	Raw Turbidity Effluent	Raw pH Effluent
No Sewage	Filtration Alone		4.97%	-31.22%	0.16	2.49	7.39
			4.27%	-78.23%	0.66	2.46	9.04
			3.33%	-15.85%	0.07	6.21	7.45
		Average	4.19%	-41.77%	0.30	3.72	7.96
		95% CI	0.93%	36.78%	0.36	2.44	1.06
	Standard		-0.28%	-91.55%	1.07	0.306	7.02
			5.26%	-93.20%	1.17	0.246	7.41
			9.09%	-91.85%	1.09	0.295	7.68
		Average	4.69%	-92.20%	1.11	0.28	7.37
		95% CI	5.33%	1.00%	0.06	0.04	0.38
	Intermediate		-1.73%	-95.35%	1.33	0.525	8.52
			-2.54%	-95.88%	1.39	0.465	8.45
			-0.58%	-95.72%	1.37	0.484	8.62
		Average	-1.61%	-95.65%	1.36	0.49	8.53
		95% CI	1.12%	0.31%	0.03	0.03	0.10
	Original		0.83%	-89.04%	0.96	0.809	7.27
			3.61%	-87.85%	0.92	0.897	7.47
			1.66%	-89.31%	0.97	0.789	7.33
		Average	2.03%	-88.73%	0.95	0.83	7.36
		95% CI	1.61%	0.88%	0.03	0.07	0.12
Sewage	Just Filtration		1.20%	-68.56%	0.50	2.27	7.59
			3.37%	-56.64%	0.36	3.3	7.66
			0.97%	-13.20%	0.06	7.56	7.26
		Average	1.85%	-46.13%	0.31	4.38	7.50
		95% CI	1.50%	32.97%	0.26	3.17	0.24
	Standard		1.47%	-88.98%	0.96	0.796	7.61
			3.73%	-95.21%	1.32	0.346	7.78
			11.60%	-94.47%	1.26	0.399	8.37
		Average	5.60%	-92.89%	1.18	0.51	7.92
		95% CI	6.02%	3.85%	0.22	0.28	0.45
	Intermediate		4.59%	-90.68%	1.03	0.709	7.75
			4.45%	-96.01%	1.40	0.304	7.74
			9.85%	-92.81%	1.14	0.547	8.14
		Average	6.30%	-93.17%	1.19	0.52	7.88
		95% CI	3.48%	3.03%	0.21	0.23	0.26
	Original		5.01%	-92.45%	1.12	0.658	7.55
			1.67%	-90.20%	1.01	0.854	7.31
			3.20%	-94.01%	1.22	0.522	7.42
		Average	3.29%	-92.22%	1.12	0.68	7.43
		95% CI	1.89%	2.17%	0.12	0.19	0.14

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